A study of the taxonomy and pathogenicity of microfungi in the roots of Waikato pasture plants.

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Nicholas William Waipara

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CONTENTS Page

ABSTRACT

A diverse and characteristic mycoflora was obtained from Waikato pasture plant root tissues. In general these fungi were not specific to host, site or soil type and although forming a natural ecological group were diverse in their systematic affinities. The fungi examined were dominated by hyphomycete and zygomycete genera, sterile fungi and coelomycetous pycnidial fungi. The sterile dark and hyaline sterile fungi isolated comprised almost 40% of isolates and a variety of methods were utilised to induce sporulation as well as separate this assemblage of amorphous isolates into 15 taxonomic groups on the basis of their morphological and physiological characters. Two sterile groups were induced to sporulate in culture and were identified as *Thozetella tocklaiensis* and a species of *Phialophora.*

Most fungal species including the sterile fungi were tested for pathogenicity to pasture species and found to be non-pathogenic root-colonising fungi, however, a minority of fungi present in Waikato pastures were pathogenic to both grass and legume seedlings and plants. Pathogenicity of these fungi was demonstrated by a series of laboratory and pot experiments where seed emergence, plant dry weight yield and root growth were reduced, while disease symptoms and root death were increased compared to the controls and those inoculated with non-pathogenic fungi. Pot trials also demonstrated that temperature and moisture could affect the pathogenicity of pasture root pathogens. The potential importance of fungi in a pastoral agricultural ecosystem is discussed.

LIST OF FIGURES

LIST OF TABLES

Chapter One Page

LIST OF ABBREVIATIONS

6PG = 6-Phosphogluconate dehydrogenase
AAT = Aspartate aminotransferase
AC = Amine-citrate
AK = Adenylate kinase
BAM = Bonner and Addicotts medium
BM1 = Basal medium 1
BM2 = Basal medium 2
BT = Browntop
CF = Cocksfoot
CK = Creatin kinase
CYA = Czapek yeast autolysate agar
CPZ = Czapek dox agar
E.C = Enzyme commission code
EST-A = Esterase
GDH = Glutamate dehydrogenase
GPD = Glyceraldehyde-3-phosphodehydrogenase
GPI = Glucose-6-phosphate isomerase
GPY = Glucose peptone yeast agar
GUS = Glutamate-ammonia ligase
G6P = Glucose-6-phosphate dehydrogenase
G25N = 25% Glycerol nitrate agar
HA = Hay agar
ICD = Isocitrate dehydrogenase
IMI = International Mycological Institute
LAP = Leucine aminopeptidase
LDH = Lactate dehydrogenase
LO = Lotus
LPDA = Laboratory potato dextrose agar
LS = Longitudinal section
LSD = Least significance difference
MDH = Malate dehydrogenase
MPI = Mannose phosphate isomerase
MEA = IMI malt extract agar
MEAG = Gams' malt extract agar
MEAP = Pitt's malt extract agar
MSM = Minimum salts medium
NT = Not tested
OA = Oat agar
PCA = Potato carrot agar
PDA = Potato dextrose agar
PEP = Peptidase
PGI = Phosphoglucoisomerase
PGM = Phosphoglucomutase
PH = Phosphate
PK = Poulik
RC = Red clover
RW = Ridgeway
RG = Perennial ryegrass
SAB = Sabouraud agar

SB = Soft brome
SC = Subterranean clover
SDG = Sterile dark group
SDW = Sterile distilled water
SED = Standard error of the deviation
SEM = Scanning electron microscopy
SEA = Soil extract agar
SHG = Sterile hyaline group
SUC = Sucrose asparagine
SV = Sweet vernal
TC = Tris-citrate
TEM = Transmission electron microscopy
TF = Tall fescue.
TI = Timothy
TS = Transverse section
TSA = Tryptic soy agar
UV = Ultra violet light
V8 = V8 juice agar
VBM = V8 broth medium
WA = Water agar
WC = White clover
WHC = Water holding capacity
YEA = Yeast extract agar
YF = Yorkshire fog

GENERAL INTRODUCTION

Since Waid (1957) investigated fungi invading roots of perennial ryegrass (*Lolium perenne* L.), there have been many studies to quantify the frequency and diversity of fungi that colonise living root tissues of pasture plants, using many techniques to obtain fungi in pure culture, or to observe mycelium directly in the root tissues. As a result, studies on pasture plants over the past 40 years have shown roots to contain a unique mixture of fungi which can invade both living and decomposing roots (Waid 1974).

Plant roots stimulate microbial growth in their vicinity, with the result that the numbers of fungi and other microbes increase up to 100 times above those away from the influence of roots (Dix and Webster 1995). This is called the rhizosphere effect, and the root soil complex where it occurs is known as the rhizosphere. Rhizospheres have been estimated to contain up to 10^6 fungi g^{-1} soil, and this is due to the release of organic substances from roots (Dix and Webster 1995). Most fungi which grow in the rhizosphere and root surface are widely distributed soil inhabiting fungi, but there are a smaller number of fungi which are confined to plant root surfaces and root tissues.

There has been a distinction made between communities of fungi which colonise root surfaces (the rhizoplane) and those which are present in cortex or vascular tissues, the latter being referred to as root-colonising , root-invading, or root-infecting fungi (Waid 1974). Root-surface fungi, or rhizosphere fungi, generally utilise root exudates and sloughed epidermal cells, while root-colonising fungi invade living or senescing plant tissue, utilising soluble and structural plant substrates, and contributing to mineralisation or decomposing plant tissues (Bowen and Rovira 1976).

Some studies have also referred to root-colonising fungi as root endophytes (Fisher *et al.* 1995a). This term is correct by the current definition given in the Dictionary of Fungi (Hawksworth *et al.* 1995), which defines an endophyte as "an organism which lives within a plant". Confusion with this term is widespread, as it has been used to refer specifically to endomycorrhizae (Kinden and Brown 1975) or to symptomless fungi living within plants (Carroll 1988, White *et al.* 1996). However, the distinction between pathogens which cause disease symptoms, opportunistic latent pathogens which produce disease only under specific environmental conditions, or saprophytic fungi that have colonised living tissues, is not always apparent. Therefore, for the purposes of this study, fungi isolated from within living roots will be referred to as root-colonising.

Radicles emerging from germinated seeds are virtually free of microorganisms (Harley and Waid 1955, Taylor and Parkinson 1961, Waid 1957) and initially root tips are sterile substrata for colonisation by fungi. As roots emerge and grow through the soil fungal colonisation is initiated, often being stimulated by the production of root exudates

and mucigel (Nelson 1990, Dix and Webster 1995). Colonisation of seedling roots takes place by the progressive lateral invasion by fungi from the surrounding soil and both rhizoplane and root-colonising fungi can reflect the soil mycoflora in which they growing (Taylor and Parkinson 1961, 1964, Thornton 1965, Waid 1974). For example, in temperate regions *Fusarium, Papulospora* and *Humicola* are typical in soils and roots, and there are differences between *Mortierella* and *Trichoderma* species present in grassland and forest roots and soil (Christensen 1989).

The World Resources Institute has defined biodiversity as the collective genes, species and ecosystems in a definable region (Castellano and Bougher 1994). Biodiversity is difficult to quantify and measure in biological systems because it embraces different processes and is manifested at the ecological, morphological and molecular levels (Hawksworth and Ritchie 1993) and characters at any of these levels can be ordered into hierarchical patterns to define taxonomic groups. Biodiversity in microfungal populations has mostly been measured by the range and abundance of species in a particular host or substrate (Fisher *et al.* 1995b, Hawksworth and Ritchie 1993, Morton and Bentivenga 1994, Sikora *et al.* 1994).

Despite the understanding of the roles of soil and root fungi in decomposition and nutrient cycling (Parkinson and Coleman 1991), little is understood concerning the relationships between fungal biodiversity, plant community dynamics and ecosystem processes (Zak and Visser 1996). The current emphasis in studies of fungal biodiversity has been on determining the taxonomic richness of various ecosystems but usually without attempting to integrate species diversity with ecosystem function (Zak and Visser 1996).

The principal objectives of this study were to evaluate the species biodiversity of root-colonising fungi in Waikato high and low fertility pastures, and to investigate the relationship of these fungi with the root health and turnover of pasture plants.

CHAPTER ONE - BIODIVERSITY AND ABUNDANCE OF ROOT-COLONISING FUNGI IN HIGH AND LOW FERTILITY WAIKATO PASTURE SITES.

"everything is everywhere and the environment selects"
Baas-Becking 1934.

1.1 INTRODUCTION

A local distribution in the soil is a well-established characteristic of root-colonising fungi (Garrett 1950, Christensen 1989, Dix and Webster 1995), which results from their poor competitive growth in soil and the distribution of host species. With increased host specialisation and narrowing of host range by some root-colonising fungi, particularly root pathogens, this distribution can become increasingly localised in contrast to the comparatively general distribution of many soil fungi. Root-inhabiting fungi may also be distributed through the soil in dead infected tissue in which fungi can persist as saprophytes, as mycelium or resting structures such as conidia or sclerotia (Singleton *et al.* 1992). Mycelium in senescent infected host tissue may remain immobilised within the substrate until encountered by suitable roots, or as with *Fusarium oxysporum,* can initiate growth within the soil and thus increase opportunities for contact with host roots.

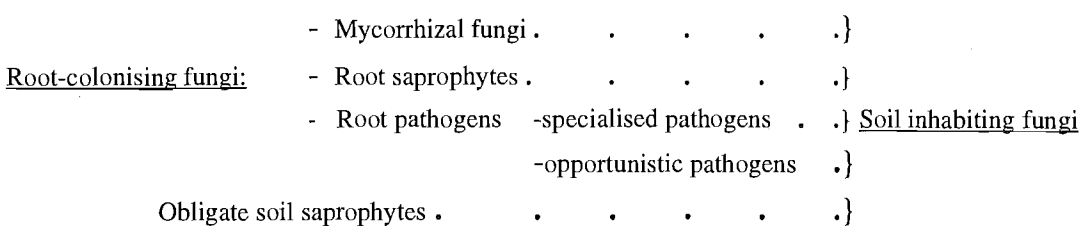
Under favourable moisture conditions rates of root growth of plants, including grasses and legumes (Cohen and Tadmor 1969), are faster than fungal growth rates. Thus roots usually can outgrow fungi (Bowen and Rovira 1976) and root tips can remain uncolonised until root growth slows. Lateral invasion of roots by primary or pioneer fungi can be by a large number of casual root invaders of which *Penicillium, Trichoderma* and zygomycetes are the most common genera isolated (Waid 1957, Taylor and Parkinson 1961, 1965, Parkinson *et al.* 1963, Dix 1964). These pioneer fungi are characterised by their rapid conidial germination and growth, and rapid primary root colonisation. Examples of this are some species of *Penicillium* whose conidia can germinate within 1 hour in the presence of roots and exudates (Bowen and Rovira 1976). Many pioneer root fungi have also been reported to be relatively insensitive to soil fungistasis, a phenomenon where conidia fail to germinate in soil (Dix 1964), and this would also enable these fungi to colonise growing roots more quickly. If inocula of root-colonising species in the soil fail to cause infection of living roots they are also unlikely to be effective in the saprophytic colonisation of senescent root tissues because of the competition from other soil inhabiting fungi (Garrett 1950). Primary fungal colonisation is followed by secondary colonisation of a succession of root-inhabiting fungi, with many of these being potential pathogens (Salt 1979). Early colonisers in this group include *Fusarium* spp., *Idriella* and *Rhizoctonia.* As the succession of fungal colonisation proceeds, sterile fungi, *Gliocladium*, and *Cylindrocarpon* spp become dominant on aging roots (Waid 1957, Taylor and Parkinson

1965, Dix and Webster 1995). Old and dying roots are also susceptible to fungal invasion by many rhizosphere fungi, such as *Volutella* spp. which do not usually colonise healthy roots (Dix 1964). Root tissues reported to be invaded by fungi include epidermis, periderm, cortex, xylem and phloem. Fungi invade root tips, root hairs primary, secondary and adventitious roots from young to senescent roots.

Associated with the cycles of root formation, function, death and decay are the many components of the soil and rhizosphere microflora, which in addition to fungi include bacteria, microalgae, protozoa and nematodes. The interactions between this multitude of soil micro-organisms and roots are still poorly understood. As soil and root fungi are important contributors to the effective function of roots and plant growth (Newman 1978), a better understanding of these interrelationships between root-colonising fungi and the associated soil microflora in the natural habitat is important.

In most roots fungi are the major component of the microflora biomass (Gams 1992a, Pankhurst and Lynch 1995), and have been classified ecologically, as saprophytes, mycorrhizal symbionts and plant parasites or pathogens. The ecological relationships between root and soil fungi was schematically outlined by Garrett (1950, Figure 1.1). These ecological groups are not independent groups but act as integrally related components of the whole soil microflora. Many fungi, such as *Fusarium culmorum,* can be characterised as both soil inhabiting and root-colonising (Burgess 1981, Farr *et al.* 1989) which is a further demonstration of the complexity of the ecological relationships between soil, roots and microorganisms.

Figure 1.1 Schematic relationships of root-colonising fungi.



(adapted from Garrett, 1950)

Garrett (1950) characterised root-colonising fungi as having an expanding parasitic phase on the living host plant, with a declining saprophytic phase after the hosts death which can be limited by competition with other microorganisms, particularly soil saprophytes. Soil-inhabiting fungi were characterised by their ability to survive

indefinitely as soil saprophytes. They competitively colonise dead plant tissue and have general distribution in the soil.

Saprophytism is defined as existence on dead organic material. Success in saprophytic competition can be attributed to several characteristics which are shared by many saprophytic fungi. Characteristics that saprophytes share are, a high growth rate, tolerance to and production of antibiotic toxins, and a capacity for rapid decomposition of dead plant tissues (Garrett 1950). Saprophytic fungi are abundant in the soil, rhizosphere and roots, stimulated by the supply of organic substrate from roots. These fungi affect the plant by altering the availability of nutrients, increasing root turnover through their role in root decomposition, and by their influence and interactions with pathogens (Newman 1978).

Soilborne fungal pathogens have important effects on plant populations by inhibiting root growth and development and causing stress through reduced nutrient uptake. Pathogens colonise roots by direct penetration, through wounds and punctures, or induce disease symptoms by secreting toxins externally from root tissues without active penetration of cells. Root pathogens can cause gross disorganisation of host root physiology and if host defence mechanisms are impaired the pathogenic invasion is likely to be then followed by root invasion by weak and opportunistic pathogens, and obligate saprophytes (Garrett 1950).

The evolution of the root infecting habit has culminated in the attainment of mycorrhizal symbioses. All agricultural soils contain mycorrhizal fungi (Mosse 1973), in particular vesicular mycorrhizal fungi, which live symbiotically in roots of most vascular plants, and these fungi increase the growth of plants by enhancing the uptake of nutrients, such as phosphorus and zinc (Mosse 1973, Powell 1976, Robson and Abbott 1987). Endomycorrhizae can increase or decrease the general resistance of plants to soilborne pathogens (Schonberg 1979), as root cells already occupied by a mycorrhizal fungus are not penetrated by parasitic fungi and are resistant to predation by nematodes. In contrast, virus diseases can increase in mycorrhizal plants, possibly due to the higher level of phosphorus uptake (Schonberg 1979).

It has been established that fungal populations of healthy or decomposing roots contrast to those of the soil and there is a specific and ubiquitous root mycoflora (Waid, 1974). The relative abundance of soil fungi which colonise roots is affected by the host plant, age of roots and fungal population present in each particular soil type (Gams 1992a). Many surveys of grassland soils and plant roots have been of temperate grasslands and have reported a low diversity of dominant fungal species in roots (Waid 1957, Kilpatrick and Dunn 1961, Gadgil 1965, Kruetzer 1972, Christensen 1989). This has also been found in previous root surveys of New Zealand pastures (Thornton 1965, Skipp and Christensen 1981, 1982, 1983, 1989a, Skipp *et al.* 1986).

New Zealand's pastoral soils developed originally under forest or natural grassland, and this natural cover has been replaced by grasslands composed of introduced temperate pasture species (McKenzie and Green 1996), which are maintained by grazing, fertiliser application and other management practices. New Zealand's pastoral agriculture relies on the sustained management of the approximate 13 million hectares of land established as permanent pastures. New Zealand pastures are composed largely of two introduced species; perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). These two species dominate the temperate high fertility pastures throughout the country, being adapted to a wide range of climate and soil type. It could be argued that this clover-ryegrass combination is the core of New Zealand's pastoral agriculture, but this pasture type can only be sustained where there is sufficient moisture and phosphate levels for plant growth. Almost all New Zealand's pastoral soils are deficient in nutrients, especially phosphorus, and some soils are also deficient in minor elements (Latch 1996), but pastures have been able to be improved by the application of superphosphate and additional trace elements.

Approximately five million hectares of sown pasture in New Zealand are on hill country which support 40% of the total livestock units (Wedderburn pers. comm.). Hill country pastures generally have low soil fertility and are heterogeneous, due to variation in moisture, temperature and soil fertility, all of which are influenced by topography (slope and aspect), and stock transfer effects (Gillingham and Bell 1977, Ledgard *et al.* 1982). As a result, hill pastures vary in plant species composition, and pure swards of white clover and ryegrass are rare (White 1990). Pastures on low fertility north-facing slopes can be dominated by annual clover and grass species which compensate for summer moisture deficits by summer seed production with autumnal germination. South-facing slopes are dominated by perennial plants which tolerate cooler temperatures. The effect of plant diversity in hill country pasture swards on the biodiversity and abundance of root-colonising fungi has previously not been investigated, as earlier surveys have been of high fertility perennial ryegrass and white clover swards (Thornton 1965, Skipp and Christensen 1983, 1989a).

Components of the soil and root mycoflora influence the sustainability of both high fertility and low fertility pastoral farming systems, pasture productivity and efficient use of management inputs (Skipp 1994). Enhancement of pasture productivity requires an understanding of the beneficial and deleterious properties of the root mycoflora and their interactions between other soil micro-organisms and host plants. The persistence and productivity of plants in perennial pastures is affected by root-fungi (Johnstone and Barbetti 1987). In particular, the decline of clover plants in both low and high fertility perennial pastures has been reported (Menzies 1973a, Williams *et al.* 1990), and the decline of white clover plants has been observed in Bay of Plenty and Waikato pastures (Watson pers. comm.). This phenomenon can seriously affect pasture productivity as

nitrogen fixation by clover increases the fertility status of the soil which enables the growth of highly productive grass species such as perennial ryegrass. The influence of root-colonising fungi on pasture persistence has yet to be investigated fully.

The first component of this study examined the biodiversity of root-colonising fungi in Waikato high and low fertility pastures, the influence of plant species on the spectrum and abundance of fungi which colonise roots, and the relative pathogenicity of these fungi to different host plants.

1.2 MATERIALS AND METHODS

A mycological study on the root-colonising fungi of Waikato pasture plants comprised three separate surveys of high and low fertility pastures, at two sites, within the Waikato region.

1.2.1 DESCRIPTION OF SURVEY SITES.

(1) Survey One: Whatawhata low fertility hill country pastures.

In survey one, root-colonising fungi were isolated from the dominant hill country pasture plant species, which were; browntop (*Agrostis capillaris* L.), white clover (*Trifolium repens* L.), sweet vernal (*Anthoxanthum odoratum* L.) and perennial ryegrass (*Lolium perenne* L.). This survey was undertaken during November 1994, at Whatawhata (20 km east of Hamilton, latitude 37°48'S). This site is representative of northern North Island hill country that typically receives a high average annual rainfall of 1500mm, but experiences warm dry summers.

(a) Survey design

Four paddocks at Whatawhata Research Centre were chosen. All four paddocks had slopes between 20-30°, with two paddocks on north facing slopes and the other two on south facing slopes. The paddocks were of low-fertility with a soil P status of 4-12 μg P/g. The soil type was Waingaro yellow brown steepland, which has a pH of 5.6. All four paddocks were rotationally grazed by cattle and sheep. Within each paddock, five plots ($2m^2$) were used for sampling. A TRASE 6050XI probe (Soil Moisture Equipment Corp.USA) was used to determine the mean percentage soil moisture at 10cm depth. The TRASE system uses time domain reflectometry, to measure instantaneously, the volumetric water content of soils. Ten readings per plot were taken and the mean calculated. A temperature probe was also used simultaneously to determine the mean soil temperature at 10cm depth for each plot (Table 1.1).

Table 1.1 Mean soil moisture and temperature (10cm depth) of Whatawhata hill country pasture plots in survey one.

	North slope1	North slope 2	South slope 1	South slope 2
% Moisture	42.9	32.0	44.8	43.9
°C Temperature	15.4	14.1	12.3	13.3

Sampling for survey one was undertaken in November 1993 (survey one)
1 = Paddock 1, 2 = Paddock 2.

Total rainfall (mm) readings from the Whatawhata weather stations in November were; South slope plots 197.5, North slope plots 210.5.

(b) Botanical composition of Whatawhata plots

Percentage plant species composition was determined for each sample plot to decide the most common pasture species and therefore the ones to be surveyed. Six classes were used to give a visual estimate of percentage coverage of plant species present in each plot.

Species composition classes;

1 = < 1%

2 = 1-4%

3 = 5-24%

4 = 25-49%

5 = 50-74%

6 = 75-100%

Table 1.2 Botanical composition of pasture plots sampled at Whatawhata.

Species	North slope 1	North slope 2	South slope 1	South slope 2
Anthoxanthum odoratum L.	4	4	4	4
Agrostis capillaris L.	3	4	3	3
Lolium perenne L.	2	2	2	3
Trifolium repens L.	2	2	3	3
Trifolium subterraneum L.	2	3	1	-
Lotus spp.	2	2	2	1
Hypochoeris radicata L.	2	1	2	1
Crepis capillaris (L.) Wallr.	1	2	2	1
Poa spp.	-	1	2	1
Holcus lanatus L.	-	-	2	2
Trifolium dubium Sibth.	1	-	-	-
Juncus acutus L.	-	1	-	1
Galium aparine L.	-	2	-	-
Bromus hordeaceus L.	1	2	-	-
Dactylis glomerata L.	1	2	-	1

1 = Paddock 1, 2 = Paddock 2

Botanical composition differed between the slopes with drought tolerant annuals, such as subterranean clover, common on north slopes and cold tolerant species, such as Yorkshire fog, common on south facing slopes (Table 1.2). The pasture composition of Whatawhata pasture plots was heterogeneous with a mixture of species (Table 1.2). The commonest species were sweet vernal and browntop, and these two species, with white clover and perennial ryegrass, were sampled.

During the survey it was found that some species in some plots had fewer plants than the sample required. When this occurred the plants were taken outside the plot but as close to the plot boundary as possible.

(2) Survey two: Ruakura high fertility dairy pastures.

Survey two aimed to isolate root-colonising fungi from the dominant dairy pasture plant species, which were; perennial ryegrass and white clover. This survey was undertaken during November 1994, at Ruakura, Hamilton, latitude 37°47'S). This site is representative of northern North Island lowland that typically receives high average annual rainfall of 2400 mm, and experiences warm moist summers.

(a) Survey design

Four paddocks at Ruakura Agricultural Research Centre were chosen. All four paddocks are of high fertility and are currently used for grazing dairy cows. Two of the paddocks were on the soil type Horotui sandy loam, which had a pH of 5.3 and a high soil P of 37.0-43.9 μg P/g. The other two paddocks were on Te Kowhai silt loam which had a pH of 5.1 and a lower soil P of 21-29.4 μg P/g. Within each paddock, five plots ($2m^2$) were used for sampling. The TRASE probe was used to determine the mean percentage soil moisture at 10 cm depth (Table 1.3). Ten readings per plot were taken and the mean calculated. A temperature probe was also used simultaneously to determine the mean soil temperature at 10 cm depth for each plot.

Table 1.3 Mean soil moisture and temperature (10 cm depth) of sample plots

	Te kowhai 1	Te kowhai 2	Horotui 1	Horotui 2
% Moisture	36.6	37.3	35.0	34.7
°C Temperature	12.6	12.2	13.7	13.1

1 = Paddock 1, 2 = Paddock 2

Total rainfall (mm) was measured at the Ruakura weather station; November 104.0.

(b) Botanical composition of Ruakura pasture plots

The same visual assessment method used at Whatawhata to determine botanical composition was applied to the Ruakura plots (Table 1.4).

Table 1.4 Botanical composition of plots sampled at Ruakura sites

Species	Te kowhai 1	Te kowhai 2	Horotui 1	Horotui 2
Lolium perenne L.	4	4	4	5
Trifolium repens L.	4	4	4	4
Taraxacum officianale Weber.	-	1	2	2
Agrostis capillaris L.	1	1	-	-
Plantago spp.	1	1	1	2
Anthoxanthum odoratum L.	2	-	-	-
Crepis capillaris (L.) Wallr.	1	1	-	-
Ranunculus repens L.	-	-	2	-
Poa spp.	1	1	1	-
Dactylis glomerata L.	1	1	-	-

1 = Paddock 1, 2 = Paddock 2

These pastures were more homogenous, particularly those on Horotui soil which were largely composed of perennial ryegrass and white clover, and it was these two species which were sampled in survey two. There was a larger diversity of plant species in the Te Kowhai pastures particularly as weeds were more common. An overall observation made while carrying out the botanical composition assessments was that plants growing on the Ruakura pasture sites were larger than those growing on the low fertility pasture plots at Whatawhata.

(3) Survey three: Whatawhata pasture sites

Survey three was designed to isolate root-colonising fungi from an additional four pasture species from the hill country pasture plots at Whatawhata. The four species chosen were; Yorkshire fog *(Holcus lanatus),* lotus *(Lotus uliginosus),* subterranean clover *(Trifolium subterraneum)* and soft brome *(Bromus hordeaceus),* which were common companion plants to the dominant species already sampled in survey one. This survey was undertaken to further investigate the host specificity of root-colonising fungi within a pasture and was undertaken in late Summer (March 1994) with the same plots at Whatawhata being sampled. Soil moisture and temperature were obtained by the same methods outlined above, and results given in Table 1.5.

Table 1.5 Mean soil moisture and temperature (10 cm depth) of Whatawhata hill country pasture plots in survey three.

	North slope1	North slope 2	South slope 1	South slope 2
% Moisture	16.3	17.0	28.1	30.7
°C Temperature	21.99	19.78	17.6	18.5

Sampling for survey three was undertaken in March 1994 (survey three).
1 = Paddock 1, 2 = Paddock 2.

Total rainfall (mm) readings from the Whatawhata weather stations in March were; South slope plots 69.0, North slope plots 67.0.

1.2.2. COLLECTION OF FIELD SAMPLES.

Five plants of each pasture species selected from each plot were sampled in each survey, and a total of 100 plants sampled for each pasture species. Plants were removed from the soil using a trowel or a 5 cm wide soil corer to a depth of at least 10 cm, and kept moist in a paper bag for isolation procedures.

1.2.3. ISOLATION OF FUNGI.

Plants were washed in running tap water for 30 minutes to remove adhering soil particles. Only roots that remained attached to identifiable plant shoots were used for isolation. Roots were then dissected from shoot material and rinsed further in sterile distilled water. Roots were surface sterilised in 1% sodium hypochlorite [NaOCl] for 5 minutes, after which they were rinsed in three changes of sterile distilled water (SDW). After drying on sterile blotting paper, white roots were cut into 2-5 mm segments and plated onto water agar (WA, appendix 1) amended with antibiotic 0.05 g/L of chloramphenicol. Only white roots were plated to avoid old and senescent roots. Plates were incubated at 20°C for 6 weeks and hyphae growing out from root segments within that time were picked off with a scalpel and subcultured onto potato carrot agar (PCA, appendix 1) for identification. The colony growth rate on PCA of each species isolated was also recorded by incubating each isolate at 25°C for 7 days. The number of root segments that were plated in each survey is outlined below;

(1) Survey one.

A total of 4000 root segments, 1000 for each plant species, were plated from plants sampled in survey one. The sampling design is summarized in Figure 1.2. Five plants were sampled from five plots, and then ten segments were dissected from each plant for isolation.

Figure 1.2 Experimental design for sampling each plant species in survey one.

{Northern -Paddock 1...-plots 1-5 -25 plants (5/plot)....10 segments/plant
-Paddock 2...-plots 1-5 -25 plants (5/plot)....10 segments/plant

ASPECT:

{Southern -Paddock 1...-plots 1-5 -25 plants (5/plot)....10 segments/plant
-Paddock 2...-plots 1-5 -25 plants (5/plot)....10 segments/plant

Number of root segments plated for each plant species:

	Total	No. of segments plated
Plant species	4	1000
Aspect	2 (North, South)	500
Paddocks	4 (2 North, 2 South)	250
Plots	20	50
Plant	100	10
Total	**4000**	**4000**

(2) Survey two.

A total of 3000 root segments were plated from plants sampled in survey two, with 750 segments from both white clover and perennial ryegrass from both soil types being plated onto WA (Figure 1.3). Fifteen root segments were dissected from each plant for isolation.

Figure 1.3 Experimental design for sampling each plant species in survey two.

{Te Kowhai -Paddock 1...-plots 1-5 -25 plants (5/plot)...15 segments/plant
-Paddock 2...-plots 1-5 -25 plants (5/plot)...15 segments/plant

SOIL TYPE:

{Horotui -Paddock 1...-plots 1-5 -25 plants (5/plot)...15 segments/plant
-Paddock 2...-plots 1-5-25 plants (5/plot)...15 segments/plant

Number of root segments plated for each plant species:

	Total	No. of segments plated
Plant species	2	1500
Soil type	2 (Te kowhai, Horotui)	750
Paddocks	4	375
Plots	20	75
Plant	100	15
Total segments	**3000**	**3000**

(3) Survey three.

A total of 400 root segments were plated onto WA in survey three, 100 segments from each plant species. The same plots and experimental design used in survey one were used again for this survey, but the segment sample size was much lower with one root segment plated from each plant (Figure 1.4).

Figure 1.4. Experimental design for sampling each plant species in survey three.

{Northern -Paddock 1...-plots 1-5 -25 plants (5/plot)....1 segment/plant
-Paddock 2...-plots 1-5 -25 plants (5/plot)....1 segment/plant

ASPECT:

{Southern -Paddock 1...-plots 1-5 -25 plants (5/plot)....1 segment/plant
-Paddock 2...-plots 1-5 -25 plants (5/plot)....1 segment/plant

Number of root segments plated for each plant species:

	Total	No. of segments plated
Plant species	4	100
Aspect	2 (North, South)	50
Paddocks	4 (2 North, 2 South)	25
Plots	20	5
Plant	100	1
Total	**400**	**400**

1.2.4. IDENTIFICATION OF FUNGAL ISOLATES.

Cultures were examined by light microscopy for identification. Some species could be identified directly on the WA isolation plates but most isolates were subcultured onto PCA or potato dextrose agar (PDA, appendix 1) for further examination. Those cultures not sporulating after four weeks growth on PCA, were subcultured onto hay agar (HA) and incubated at room temperature in daylight. Isolates still not sporulating after 6 weeks on HA were classed as sterile cultures and were stored for further morphological examination as outlined in chapter two.

Several methods and taxonomic keys can be used for identification of fungal species. One authority and identification protocol was generally used in this study to allow consistency in results, and these methods used for the classification of frequently isolated genera are listed below;

(1) *Fusarium*

Each *Fusarium* isolate was inoculated onto two plates of laboratory potato dextrose agar (LPDA, appendix 1). One plate was incubated at 25°C, the other at 30°C, and colony diameters were measured after 3 days. Each isolate was also inoculated once onto HA and incubated in daylight at room temperature until sporulation occurred. Colony diameters and fungal morphology on HA were used to identify each isolate according to the identification key of Burgess *et al.* (1988).

(2) *Penicillium*

Each *Penicillium* isolate was plated onto three media for identification, Czapek yeast autolysate agar (CYA, appendix 1), Pitt's malt extract agar (MEAP, appendix 1), and 25% glycerol nitrate agar (G25N, appendix 1). To prevent formation of colonies from stray spores, inoculation of all penicillia was made from a semisolid suspension of conidia. 0.5 ml of melted WA (0.15%) and 1 drop of 'Tween 80' were dispensed into small bijou bottles, autoclaved and inoculated with loop of conidia from a sporulating PCA culture. After mixing the conidia into the media, plates were inoculated by stabbing a needle into the suspension and then onto the inoculation plates. Inoculation was made at three points on one Petri dish per isolate. Each isolate was inoculated in following method;

(i) Once on MEAP which was incubated at 25°C,

(ii) Three times on plates of CYA, which were incubated at three temperatures, 5°C, 25°C and 37°C.,

(iii) Once on G25N and incubated at 25°C.,

Colony diameters are measured after 7 days incubation at all temperatures. Colony growth at 5°C, was classed as: conidial germination absent, germination, microcolony formation and macrocolony formation. Colony growth rates were used in conjunction with fungal morphology on MEAP, as diagnostic characters to identify each isolate using the identification key of Pitt (1979).

(3) *Trichoderma*

Isolates were inoculated onto oat agar (OA, appendix 1) and incubated at 20°C for 5 days to measure colony growth diameter. Conidiophore morphology was observed by inoculating isolates on HA and incubating in daylight at room temperature. The identification key of Rifai (1969) was used to classify each species.

(4) *Chaetomium* and other Ascomycetes

Isolates of the genus *Chaetomium* were inoculated onto OA and incubated at 20°C for ten days to measure colony diameter, which is a diagnostic character used in the identification keys given in (Domsch *et al.* 1980), and (Hawksworth 1993). Both keys were used to identify these species. This genus and other ascomyceteous isolates were inoculated onto HA and incubated in daylight at room temperature until ascomata were produced at the agar-hay piece interface.

(5) *Aspergillus*

Isolates were inoculated from semisolid suspensions onto Czapek dox agar (CPZ, appendix 1) and incubated at 25°C for 7-14 days to measure colony growth rates. Cultural morphology was observed on PCA and the identification key of Raper and Fennell (1965) used to identify all isolates.

(6) *Acremonium*

All isolates of this genus were inoculated onto malt extract agar Gams recipe (MEAG, appendix 1), and incubated at 20°C for 10 days to determine colony growth rates. Morphology was also examined on HA. The identification key of Gams (1971a) was used to identify all species. This key was also used to identify other monilaceous hyphomycetes such as *Aphanocladium, Verticillium* and *Tolypocladium.*

(7) *Mortierella* & other Zygomycetes

Each isolate was inoculated onto MEAG and incubated at 20°C for 5 days to assess colony growth rate. Cultural morphology was examined on HA and the identification keys of Gams (1977) and Domsch *et al.* (1980) used to differentiate each isolate into species.

(8) *Dactylaria* and *Arthrobotrys*

Isolates from these two genera were identified according to key of De Hoog and Van Oorschot (1985) and all isolates were examined by light microscopy on PCA, HA and OA for growth rates and morphology.

(9) *Bipolaris & Drechslera*

To differentiate between the genera of *Bipolaris, Drechslera,* and *Exserohilum* the mode of conidial germination must be observed. Conidia from sporulating cultures are mixed with a sterile loop in 5 ml of SDW from which 0.5 ml of this conidial suspension of each isolate was pipetted onto a WA plate and incubated at 20°C for 12 hours. Each isolate was then inoculated onto; MEA (standard IMI recipe, appendix 1), and incubated at 20°C to determine colony growth rates. Fungal morphology was examined on HA after 2-3 weeks growth at room temperature. Isolates were identified using the key of Sivanesan (1987).

(10) Yeasts

Yeast species are generally differentiated by a combination of morphological and physiological properties under standard conditions. Physiological properties are primarily used to differentiate and describe yeast species and strains. The fermentation and assimilation of nitrogen and carbon compounds are the most useful and important physiological characteristics used for identification (Kreger-van Rij 1984). The basidiomycetous yeast-like species, *Aureobasidium pullulans,* was able to be identified by morphological examination on PCA and so was not included in these tests.

(a) Morphological properties.

Each yeast isolate was streaked onto glucose peptone yeast extract agar (GPY, appendix 1), and incubated at 20°C for 5 days to examine yeast morphology by light microscopy. Apart from one isolate, all isolates produced arthrospores and blastospores on GPY, which differentiated these isolates into the *Trichosporon* genus.

(a) Physiological properties.

(i) N-auxanographic test to determine nitrogen assimilation.

The assimilation of nitrogen by each isolate was determined by inoculating each isolate with a loop into testubes containing 5 ml of three different basic medium broths (BM1, appendix 1). The first BM1 broth had no added nitrogen (a negative control), the second had potassium nitrate [KNO_3] added, and the third had Ammonium nitrate [$(NH_4)_2NO_3$] added, which was a positive control as all yeasts assimilate this nitrogen compound. All inoculated broths were incubated at 20°C in darkness and assessed after 3 weeks, where growth in the broth was determined as positive assimilation.

(ii) Carbon fermentation.

The presence or absence of fermentation was determined by the production of carbon dioxide from carbohydrates. Each isolate was inoculated into plugged sterile test tubes containing 5 ml of glucose peptone yeast broth (GPY broth, appendix 1) with an inverted glass Durham tube inserted into the tube. Inoculated tubes were incubated at 25°C and assessed after 14 days, with positive carbon fermentation being determined by the visible production of gas in the Durham tubes.

(iii) C-auxanographic test to determine carbon assimilation.

A number of growth tests were undertaken to determine the ability or inability of these isolates to utilise carbon compounds. Aliquots of yeast suspensions were prepared by adding two loopfuls of each isolate to bijou bottles containing;10 ml of SDW and 2 ml yeast extract. A 2 ml drop of yeast suspension was added into three glass Petri dishes for each isolate and then 20 ml of carbon deficient basal medium (BM2, appendix 1) cooled to 40°C was poured on top. The suspension was mixed through the media by a sterile loop and left to solidify. At marked places on the plate 1 g of each carbon compound was added with a spatula. Nine carbon compounds, used to differentiate *Trichosporon* species, were tested for assimilation: lactose, maltose, cellobiose, erythritol, ribitol, xylose, melibiose, insitol and glucose (a positive control as all yeasts can assimilate this compound). After 5

days incubation at 20°C, each plate was flooded with Grams iodine to stain any starch compounds. Results were recorded as positive (stained) or negative (unstained), which were then applied to Kreger-van Rij's (1984) yeast identification key.

(11) *Rhizoctonia*

Determination of the number of nuclei in vegetative hyphal cells is an important process in the identification of *Rhizoctonia* species. Isolates were grown on WA at 20°C for 5 days, after which a drop of 5% trypan blue in lactophenol was placed directly on hyphae growing across the agar surface. A cover slip was placed over the stained hyphae and examined at 400x by light microscopy. Nuclei were stained dark blue and this character, along with cultural morphology, was used to identify this genus to species using the identification keys of Sneh *et al.* (1991) and Hawksworth (1993).

(12) Other Deuteromycetes

Remaining isolates were inoculated onto PCA and/or HA and incubated at 20°C until sporulation occurred. Identification keys for classifying soil fungi were then used to identify described species (Barron 1968, Domsch *et al.* 1980, Ellis 1971, Ellis 1976, Hawksworth 1993).

(13) Bacteria

Despite amending the isolation media with the antibiotic chloramphenicol, a number of bacterial isolates were observed growing from root segments. There were two species that were consistently growing from these segments and so were identified with the Biolog MicroStation bacterial identification system. This system is a standardised micromethod, using 95 biochemical tests to identify a broad range of bacteria.

Bacteria were streaked into pure culture on tryptic soy agar (TSA, appendix 1). Colonies grown on TSA for 12 hours at 25°C, were swabbed into tubes containing 20 ml of sterile saline solution (appendix 1). Cell density of the solution was measured with a Biolog turbidimeter which measured turbidity, and is a measure of cell density. Once the cell density was approximately 3x 10^8 cells/ml, the cell suspension of each isolate was inoculated into Biolog microplates using a pipette. All wells of the microplate were filled with 150 µl before being incubated at 30°C for 12 hours. Biolog microplates test the ability of a micro-organism to utilise or oxidise 95 preselected carbon sources. Tetrazolium violet was used as a redox dye to colormetrically indicate the utilisation of the carbon sources, a purple colour in a well indicated a positive utilisation of the carbon source. After the microplates had been incubated, the purple pattern of the wells was keyed into the Biolog computer programme to find an adequate match to allow identification of each isolate.

1.2.5. CULTURE MAINTENANCE.

Three methods of storing isolates were used for the duration of the project and are outlined below (1-4);

(1) Culture slope

Universal bottles containing 8ml slopes of PCA, were inoculated with a 3 mm plug of fungus and incubated at room temperature until the slope surface was colonised by fungal growth. Bottles were kept refrigerated at 4°C until required.

(2) Sterile water storage

Bijou bottles containing 5 ml of SDW were autoclaved, and then inoculated with 10 fungal plugs (3 mm) from seven day old cultures grown on PCA (Smith and Onions 1994). This method was also used for storing fungi, particularly *Trichoderma* isolates, as spore suspensions. Cultures sporulating on OA or PCA were scraped off with a wire loop and inoculated into the bottles. Bottles were held at 4°C until required.

(3) Frozen storage

Important isolates that were required for pathogenicity assessment and other pot trials were also kept in frozen storage to ensure survival in culture. Nunc cryo tubes containing a 10% glycerol solution were autoclaved and inoculated with five 3 mm PCA fungal culture plugs. The tubes were then placed in a freezer at -20°C for 1 hour before being put in a -80°C freezer for long term storage.

(4) Bacterial storage

Colonies grown for 24hr on TSA were looped into bijou bottles containing 4 ml of 0.1M magnesium sulphate solution (appendix 1) and held at 4°C until required.

1.3 RESULTS

A total 7232 fungal isolates were obtained from the 7400 root segments plated in all three surveys with a total of 94.3% of root segments colonised by fungi. There was a higher percentage of root segments colonised by fungi in survey one (96%) than the other two surveys with only 80% of segments being colonised in survey three (Table 1.6). Despite the isolation media being amended with antibiotic, 71 root segments in surveys one and two were found to be colonised by bacteria. The remaining 353 segments were uncolonised, or colonised by organisms which could not be isolated by this isolation method, however this uncolonised component was very small comprising 4.8% of the total segments plated. There were 121 fungal species identified from the three surveys (Appendices 2 & 3), and over half (69 species) of these were recorded from both Whatawhata and Ruakura plots.

Table 1.6 Total number of root segments in all surveys that were colonised by fungi, bacteria or remained uncolonised.

	Survey one	Survey two	Survey three	Total
No. of segments plated	4000	3000	400	**7400**
No. of fungi isolated	4023	2876	333	**7232**
No. of segments colonised by fungi	3841	2815	320	**6976**
% of root segments colonised	96%	93.8%	80%	**94.3%**
No. of bacteria isolated	17	54	0	**71**
No. of uncolonised segments	142	131	80	**353**

All the major fungal taxonomic groups were represented in the surveys, however deuteromycetous fungi were the most common (Table 1.7), with a total 6758 isolates. Sterile fungi were also common, comprising 39% of isolates. The remaining three groups, ascomycetes, basidiomycetes and zygomycetes, were all uncommon on pasture roots and collectively totaled less than 10%. There were 21 isolates which remained unidentified, but these isolates only comprised 0.3% of fungi (Table 1.7). Deuteromycetous fungi were ubiquitous on the roots of pasture plants with 99 species being identified . This group was composed of three large taxonomic classes, Hyphomycetes (3772 isolates), Agonomycetes (Sterile fungi 2824 isolates) and Coelomycetes (162 isolates).

Fusarium was the most frequently isolated hyphomycete genus with over 16% of isolates from ten species being recorded, and *Fusarium oxysporum* was the most common species to be identified in this study. Species of the hyphomycete genera; *Codinaea, Penicillium* and *Trichoderma* were also frequently isolated in all surveys. *Penicillium* was the most diverse group with 19 species being identified. Three yeast species were also isolated (Table 1.7), and these comprised 1% of fungi that were isolated. Nine species each of zygomycetes and ascomycetes were recorded, and one species of *Rhizoctonia*, a basidiomycete, making this class the least common group of fungi obtained in this study.

Although over 100 species were identified, most were uncommon, being isolated with low frequencies of less than 1% (Table 1.7 and Appendix 2). There were only 14 species which had isolation frequencies over 1% of the total, but these few common species collectively comprised almost 40% of the fungi isolated. Appendix 3 provides a complete species list, an index of the isolation site and host, and previous host records of all the fugal species recorded in this survey.

Table 1.7 Fungi isolated from plants at Whatawhata and Ruakura pastures.

Fungi:	No. of species	Total count	Percentage (%)
Deuteromycetes:	**99**	**6758**	**93.4%**
A) Hyphomycetes:	**98**	**3772**	**52.2%**
Acremonium **spp.**	5	123	1.7%
Aspergillus **spp.**	4	33	0.5%
Codinaea fertilis	1	709	9.8%
Cylindrocarpon destructans	1	139	1.9%
Cylindrocladium scoparium	1	92	1.3%
Dactylaria acerosa	1	75	1.0%
Fusarium:	**10**	**1162**	**16.1%**
F. avenaceum	1	102	1.4%
F. crookwellense	1	104	1.4%
F. culmorum	1	73	1.0%
F. oxysporum	1	818	11.3%
F. solani	1	41	0.6%
Other *Fusarium* **spp.**	5	24	0.3%
Gliocladium **spp.**	3	165	2.3%
Mariannaea elegans	1	36	0.5%
Idriella bolleyi	1	50	0.7%
Paecilomyces **spp.**	3	113	1.6%
Penicillium:	**19**	**219**	**3.0%**
P. chrysogenum	1	35	0.5%
P. janthinellum	1	82	1.1%
P. simplicissimum	1	60	0.8%
Other *Penicillium spp.*	16	42	0.6%
Periconia macrospinosa	1	124	1.7%
Trichoderma:	**7**	**373**	**5.2%**
T. hamatum	1	145	2.0%
T. harzianum	1	54	0.7%
T. koningii	1	62	0.9%
T. polysporum	1	90	1.2%
Other *Trichoderma* **spp.**	3	22	0.3%
Verticillium **spp.**	5	90	1.2%
Other Hyphomycete spp.	34	268	3.7%
B) Coelomycetes	3	163	2.3%
Colletotrichum **spp.**	2	27	0.3%
Pycnidial fungi	*	**135**	**1.9%**
C) Agonomycetes	*	**2824**	**39.0%**
Sterile dark fungi	*	**2216**	**30.6%**
Sterile hyaline fungi	*	**608**	**8.4%**

Fungi:	No. of species	Total count	Percentage (%)
Ascomycetes:	**9**	**158**	**2.2%**
Bimuria novae zelandiae	1	81	1.2 %
Chaetomium	3	36	0.5%
Preussia aemulans	1	24	0.3%
Other Ascomycete spp.	4	17	0.2%
Basidiomycetes	**1**	**24**	**0.3%**
Yeasts and allied species	**3**	**75**	**1.0%**
Zygomycetes:	**9**	**196**	**2.7%**
Gongronella butleri	1	51	0.7%
***Mortierella* spp.**	6	102	1.4%
***Mucor* spp.**	2	43	0.6%
Unidentified fungi	*	21	0.3%
TOTAL	**121**	**7232**	

* The number of species of pycnidial, sterile and unidentified isolates was not determined.

Appendices 2 and 3 provide complete lists of all species that were identified in the study.

Species of pycnidial fungi were the main group of coelomycetes isolated in this study. These fungi are comprised of several genera which produce asexual spores in pycnidia. Identification of these fungi to species require specialist identification techniques (Sutton 1980). As they comprised almost 2% of isolates (Table 1.7), further work should be undertaken to ascertain which species are numerically important in Waikato pastures.

Colony growth of fungal species on PCA, indicated that a range of species with different growth rates were obtained (Table 1.8). Fungi with medium growth rates were the most frequently isolated in all surveys. Slow growing fungi were also obtained in large numbers which showed they had not been overwhelmed by fungi with more competitive and rapid growth rates (35% of total isolates). A complete list of the average colony growth rates is provided in Appendix 4.

Table 1.8 Total number and percentage of fast, medium and slow growing fungi isolated from root segments and grown on PCA at 20°C after 14 days.

	Survey one	Survey two	Survey three	Total
No. of isolates tested	4003	2856	330	**7189**
No. of fungi with rapid growth[1]	1313 (32.7%)	1106 (38.7%)	105 (31.8%)	**2524 (35.1%)**
No. of fungi with medium growth[2]	1904 (47.5%)	1440 (50.4%)	200 (60.6%)	**3544 (49.3%)**
No. of fungi with slow growth[3]	791 (19.8%)	305 (10.7%)	25 (7.6%)	**1121 (15.6%)**

1= (Colony diameter >50mm/14 days)

2= (Colony diameter 20-50mm/14 days)

3= (Colony diameter <20mm/14 days)

I. SURVEY ONE.

A total of 4023 isolates were obtained from a total of 4000 root segments in survey one (Tables 1.9 & 1.10). The number of fungal isolates obtained from browntop and ryegrass exceeded the total number of segments that were plated (Table 1.9), as numerous

segments were often colonised by more than one fungus, and 96% of root segments were colonised by at least one fungus. A total of 17 bacteria were also obtained from three of the plants and these were all identified as *Pseudomonas corrugata*, while no fungi or bacteria could be isolated from 142 segments (Table 1.9).

Table 1.9 Total number of root segments in Survey one that were colonised by fungi, bacteria or remained uncolonised.

	Sweet vernal	Browntop	Clover	Ryegrass	Total
No. of segments plated	1000	1000	1000	1000	**4000**
No. of fungi isolated	946	1096	948	1033	**4023**
No. of segments colonised by fungi	929	990	940	982	**3841**
% of root segments colonised	92.9%	99.0%	94.0%	98.2%	**96%**
No. of bacteria isolated	2	-	14	1	**17**
No. of uncolonised segments	69	10	46	17	**142**

Over 100 species of fungi were identified from the four plant species sampled in survey one (Appendix 2). Each pasture species had similar numbers of fungal species present on roots ranging from 65 on browntop, to 61 on both sweet vernal and ryegrass (Table 1.10). Hyphomycete fungi were the most common group of fungi found on roots at Whatawhata, with a total of 1857 isolates being identified. *Fusarium oxysporum* (9.7%) and *Codinaea fertilis* (8.2%) were the most commonly identified hyphomycete species, as they occurred on all plants. Species from common soil hyphomycete genera; *Acremonium, Cylindrocarpon, Dactylaria, Fusarium, Gliocladium, Paecilomyces, Penicillium, Trichoderma* and *Verticillium* were frequently identified from root segments of all plants (Table 1.10). Sterile fungi (agonomycetes) were also an important component of the pasture root mycoflora totaling 39% of all isolates and sterile dark isolates were obtained with twice the frequency of sterile hyaline isolates. The remaining groups of fungi; ascomycetes, coelomycetes, yeasts, basidiomycetes and zygomycetes were relatively uncommon, each comprising less than 2% of isolates. Overall, the mycoflora of all four plants were similar as over 60% of fungi were found on all plant species.

Table 1.10 Total number and percentage of fungal species isolated from surface sterilised root segments of sweet vernal, browntop, white clover and perennial ryegrass from Whatawhata hill country pastures in survey one.

PLANT:	Browntop	Ryegrass	Sweet vernal	Clover	Total count	Total %
FUNGAL SPECIES:						
Hyphomycetes:	**424**	**499**	**335**	**599**	**1857**	**46.2**
-*Acremonium* spp.	23	17	15	23	**78**	1.9
-*Codinaea fertilis*	62	78	56	134	**330**	8.2
-*Cylindrocarpon destructans*	6	15	1	26	**48**	1.2
-*Cylindrocladium scoparium*	0	0	0	31	**31**	0.8
-*Dactylaria acerosa*	13	16	20	18	**67**	1.7
-*Fusarium* spp.*:	114	129	65	195	**503**	12.5
* *F. avenaceum*	2	1	9	27	39	1.0
* *F. crookwellense*	7	12	6	14	39	1.0
* *F. culmorum*	1	4	1	3	9	0.2
* *F. oxysporum*	98	102	42	147	389	9.7
* *F. solani*	4	5	1	2	12	0.3
* Other *Fusarium* spp.	2	5	6	2	15	0.4
-*Gliocladium* spp.	15	39	18	29	**101**	2.5
-*Paecilomyces* spp.	21	18	14	17	**70**	1.7
-*Penicillium* spp.	70	49	21	23	**163**	4.0
-*Periconia macrospinosa*	7	6	29	9	**51**	1.3
-*Trichoderma* spp.	42	66	22	36	**166**	4.1
-*Verticillium* spp.	12	15	8	13	**48**	1.2
-Other Hyphomycete spp.	39	51	66	45	**201**	5.0
B) Coelomycetes	**12**	**12**	**27**	**25**	**76**	**1.9**
C) Agonomycetes:						
-Sterile dark fungi	438	390	408	211	**1447**	28.5
-Sterile hyaline fungi	178	80	132	31	**421**	10.5
Ascomycetes:	**10**	**10**	**13**	**22**	**55**	**1.4**
-*Bimuria novae zelandiae*	0	0	0	19	19	0.4
Basidiomycetes	**7**	**6**	**4**	**5**	**22**	**0.5**
Yeasts and allied species	**2**	**13**	**14**	**21**	**50**	**1.2**
Zygomycetes	**25**	**23**	**13**	**15**	**76**	**1.9**
TOTAL	**946**	**1096**	**948**	**1033**	**4023**	
Total no. of species identified	**61**	**65**	**63**	**61**	**101**	

Appendix 2 contains the complete isolation frequency of all fungal species.
* *Fusarium* species

Sterile fungi were an important component of the root mycoflora of all the plants sampled in this survey (Table 1.11). Both sterile dark and hyaline fungi were the commonest fungi isolated from all root segments. *Fusarium oxysporum* and *Codinaea fertilis* were also frequently identified from all plants, particularly from white clover roots. Other common species were *Trichoderma polysporum, Cylindrocarpon destructans* and *Gliocladium roseum* on ryegrass (Table 1.11), *Penicillium janthinellum* from browntop and *Periconia macrospinosa* from sweet vernal. The mycoflora of clover was distinct from the three grasses as there were less sterile isolates obtained, instead species such as; *Bimuria novae zelandiae, Cylindrocladium scoparium, Cylindrocarpon destructans* and *Fusarium avenaceum,* were more frequently isolated from clover roots than from the grasses.

Table 1.11 The rank, number and percentage isolation of the ten most common fungi obtained from pasture plants in survey one.

Sweet vernal	Rank	No.	%	Browntop	Rank	No.	%
Sterile dark fungi	**1**	144	15.2	**Sterile hyaline group 3**	**1**	125	11.4
Sterile dark group 6	**2**	86	9.1	**Sterile dark group 4**	**2**	121	11.0
Sterile hyaline group 3	**3**	84	8.9	***Fusarium oxysporum***	**3**	98	8.9
Sterile dark group 5	**4**	83	8.8	**Sterile dark fungi**	**4**	94	8.6
Codinaea fertilis	**5**	56	5.9	**Sterile dark group 6**	**5**	75	6.8
Fusarium oxysporum	**6**	42	4.4	**Sterile dark group 5**	**6**	69	6.3
Sterile dark group 7	**7**	30	3.2	**Sterile dark group 7**	**7**	64	5.8
Periconia macrospinosa	**8**	29	3.1	***Codinaea fertilis***	**8**	62	5.7
Sterile hyaline group 1	**9**	25	2.6	**Sterile hyaline fungi**	**9**	43	3.9
Pycnidial fungi	**10**	23	2.4	***Penicillium janthinellum***	**10**	35	3.2
White clover				**Perennial ryegrass**			
	Rank	No.	%		Rank	No.	%
Fusarium oxysporum	**1**	394	17.2	**Sterile dark group 5**	**1**	309	12.1
Codinaea fertilis	**2**	363	15.8	***Fusarium oxysporum***	**2**	238	9.3
Sterile dark group 5	**3**	208	9.1	**Sterile dark fungi**	**3**	206	8.1
Sterile dark fungi	**4**	105	4.6	***Codinaea fertilis***	**4**	177	6.9
Cylindrocladium scoparium	**5**	85	3.7	**Sterile dark group 7**	**5**	167	6.5
Bimuria novae zelandiae	**6**	81	3.5	**Sterile dark group 6**	**6**	120	4.7
Cylindrocarpon destructans	**7**	68	3.0	**Sterile hyaline fungi**	**7**	100	3.9
Gliocladium roseum	**8**	60	2.6	***Trichoderma polysporum***	**8**	65	2.6
Fusarium avenaceum	**9**	58	2.5	***Cylindrocarpon destructans***	**9**	64	2.5
Fusarium crookwellense	**10**	49	2.1	***Gliocladium roseum***	**10**	62	2.4

The influence of aspect on the diversity of frequently isolated fungi appeared to be minimal, as most dominant species were isolated from roots of both north and south facing slopes (Table 1.12). There was some difference in the abundance of fungi isolated from the two aspects. Total fungal isolation was higher from south facing plots with over 2000 fungi recorded (Table 1.12), and this may have corresponded to the higher number of species being isolated from these plots. There were also some differences in the abundance of some dominant fungi between the two slopes. For example, *Codinaea fertilis* and *Fusarium* spp. were more commonly isolated from the dry north facing plots, and *Cylindrocladium scoparium, Acremonium* and sterile fungi were more abundant on south facing slopes. However, these differences were the exception as most dominant fungi, such as *Penicillium* and *Trichoderma,* were isolated with similar frequencies from both slopes.

Table 1.12 Total number and percentage of fungal species isolated from North and south facing pastures at Whatawhata.

Aspect	Northern slopes		Southern slopes	
Fungal Species	**Count**	**%**	**Count**	**%**
Hyphomycetes:	**1000**	**51.4**	**876**	**42.2**
-*Acremonium* **spp.**	28	1.4	50	2.1
-*Codinaea fertilis*	179	9.2	151	7.3
-*Cylindrocarpon destructans*	23	1.2	25	1.2
-*Cylindrocladium scoparium*	2	0.1	29	1.4
Dactylaria acerosa	39	2.0	28	1.3
-*Fusarium* **spp.*:**	322	16.6	181	8.7
**Fusarium oxysporum*	263	13.5	126	6.1
-*Gliocladium* **spp.**	44	2.3	57	2.7
-*Paecilomyces* **spp.**	43	2.2	27	1.3
-*Penicillium* **spp.**	81	4.2	83	4.0
-*Periconia macrospinosa*	34	1.7	17	0.8
-*Trichoderma* **spp.**	80	4.1	85	4.1
-*Verticillium* **spp.**	23	1.2	25	1.2
-Other Hyphomycete spp.	102	5.2	118	5.7
B) Coelomycetes	**30**	**1.5**	**46**	**2.2**
C) Agonomycetes:	**802**	**41.2**	**1066**	**51.3**
-Sterile dark fungi	632	32.5	811	39.0
-Sterile hyaline fungi	170	8.7	255	12.3
Ascomycetes:	**31**	**1.6**	**24**	**1.2**
Basidiomycetes	**10**	**0.5**	**12**	**0.6**
Yeasts and allied species	**31**	**1.6**	**19**	**0.9**
Zygomycetes	**41**	**2.1**	**35**	**1.7**
Total	**1945**	**100**	**2078**	**100**
No. of species identified	**77**		**83**	

Appendix 5 contains the complete isolation frequency of all fungal species from both slopes. * *Fusarium* species

II. SURVEY TWO

A total of 2876 fungi were isolated from 2815 root segments (Table 1.13), and the remaining 185 plated segments were colonised by bacteria (54 segments) or were uncolonised (131 segments). Four bacterial species were identified, *Pseudomonas corrugata* (39 isolates), *P. tolaasii* (12 isolates), *Spingomonas paucimobilis* (one isolate) and *Xanthomonas campestris* var *xanthomonas* (two isolates). More fungi were isolated from ryegrass roots than clover roots (Table 1.13), however over 90% of root segments were colonised by fungi.

Table 1.13 Total number of root segments in Survey one that were colonised by fungi, bacteria or remained uncolonised.

	Ryegrass	Clover	Total
No. of segments plated	1500	1500	**3000**
No. of fungi isolated	1526	1350	**2876**
No. of segments colonised by fungi	1465	1350	**2815**
% root colonisation by fungi	97.7%	90.2%	**93.8%**
No. of bacteria isolated	16	38	**54**
No. of uncolonised segments	19	112	**131**

A total of 86 species were isolated from the roots of clover and ryegrass plants sampled in survey two (Table 1.14). Most of these species were the same as those identified in survey one, indicating the root mycofloras of pasture plants were the same at both sites. There were only 12 species identified in survey two that were absent from survey one, these were, *Aspergillus glaucus, Botryosporium, Mortierella baineri, Tetraploa aristata, Thielaviopsis basicola, Penicillium griseofulvum, Penicillium islandicum, Phymatotrichum omnivorum, Sordaria fimicola, Sporothrix schenckii, Pestalotia* and *Pyrenophora,* (Appendix 2), and these species were all uncommon as they comprised fewer than 1% of the total fungi isolated. Species from common soil genera, *Acremonium, Cylindrocarpon, Fusarium, Gliocladium, Mortierella, Paecilomyces, Penicillium Trichoderma* and *Verticillium,* were frequently identified from root segments (Table 1.14). Sterile fungi were also very common (Table 1.14), with pigmented and hyaline isolates comprising over 30% of the fungi isolated. Three groups of fungi, ascomycetes, coelomycetes and zygomycetes each comprised 3-4% of isolates, with the remaining two groups; yeasts and basidiomycetes being rarely isolated. There were few differences between the root mycofloras of clover and ryegrass at Ruakura, although the mycoflora of ryegrass was more diverse with eight additional species being identified.

Table 1.14 Total number and percentage of fungal species isolated from surface sterilised root segments of perennial ryegrass and white clover from Ruakura dairy pastures.

Pasture plant	Ryegrass	Clover	Total	Total
FUNGAL SPECIES:			count	%
Hyphomycetes:	664	850	1514	52.6
-*Acremonium* spp.	24	21	45	1.6
-*Codinaea fertilis*	99	229	328	11.4
-*Cylindrocarpon destructans*	49	42	91	3.2
-*Cylindrocladium scoparium*	6	54	60	2.1
-*Fusarium* spp.*:	250	343	593	20.6
* *F. avenaceum*	25	31	56	1.9
* *F. crookwellense*	28	35	63	2.2
* *F. culmorum*	47	10	57	2.0
* *F. oxysporum*	136	247	383	13.3
* *F. solani*	12	17	29	1.0
* Other *Fusarium* spp.	2	3	5	0.2
-*Gliocladium* spp.	26	33	59	2.1
-*Idriella bolleyi*	18	7	25	0.9
-*Paecilomyces* spp.	28	8	36	1.3
-*Penicillium* spp.	26	9	35	1.2
-*Periconia macrospinosa*	17	10	27	0.9
-*Trichoderma* spp.	112	65	177	6.2
-*Verticillium* spp.	10	29	39	1.4
-Other Hyphomycete spp.	86	47	133	4.6
B) Coelomycetes	39	38	79	2.7
C) Agonomycetes:				
-Sterile dark fungi	511	234	745	25.9
-Sterile hyaline fungi	118	51	169	5.9
Ascomycetes:	35	66	103	3.4
-*Bimuria novae zelandiae*	0	62	62	2.2
Basidiomycetes	1	1	2	<0.1
Yeasts and allied species	7	18	25	0.9
Zygomycetes	62	45	107	3.7
TOTAL	1526	1350	2876	
Total no. of species isolated	76	62	86	

Appendix 2 contains the complete isolation frequency of all fungal species.
* Fusarium spp.

The commonest fungi found on plants sampled in survey two were *Fusarium oxysporum, Codinaea fertilis* and sterile dark fungi, which were the same fungi with high isolation rankings in survey one. The mycoflora of white clover and ryegrass differed, as sterile fungi were more common on ryegrass roots than on clover roots, which were instead colonised by species such as; *Bimuria novae zelandiae, Cylindrocladium scoparium* and *Fusarium crookwellense* (Table 1.15). The ascomycete, *B. novae zelandiae,* was host specific to white clover while another ascomycete, *Preussia aemulans* was only recorded from ryegrass roots. The isolation frequency of both *F. oxysporum* and *C. fertilis* from clover roots was twice that of ryegrass roots. *Trichoderma polysporum* was common on ryegrass roots (55 isolates identified) but was uncommon on clover roots, with only five isolates being recorded. *Fusarium culmorum* was frequently encountered on ryegrass roots

from Ruakura which contrasts to its rare occurrence at Whatawhata. Despite these differences, the two mycofloras were similar, as 65 of 100 species and all sterile groups, occurred on both pasture plants (Appendix 2).

Table 1.15 The rank, number and percentage isolation of the ten most common fungi obtained from pasture plants in survey two.

White clover				Perennial ryegrass			
	Rank	No.	%		Rank	No.	%
Fusarium oxysporum	1	247	18.3	**Sterile dark group 5**	1	213	14.0
Codinaea fertilis	2	229	17.0	*Fusarium oxysporum*	2	136	8.9
Sterile dark group 5	3	102	7.6	**Sterile dark fungi**	3	111	7.27
Bimuria novae zelandiae	4	62	4.6	**Sterile dark group 7**	4	109	7.1
Sterile dark fungi	5	58	4.3	*Codinaea fertilis*	5	99	6.5
Cylindrocladium scoparium	6	54	4.0	**Sterile hyaline fungi**	6	60	3.9
Cylindrocarpon destructans	7	42	3.1	*Trichoderma polysporum*	7	55	3.6
Fusarium crookwellense	8	35	2.6	*Cylindrocarpon destructans*	8	49	3.2
Pycnidial fungi	8	35	2.6	**Sterile hyaline group 1**	8	49	3.2
Fusarium avenaceum	9	31	2.3	*Fusarium culmorum*	9	47	3.1
Gliocladium roseum	9	31	2.3	**Pycnidial fungi**	10	33	2.2

There were few differences between the fungi obtained from roots sampled from the two Ruakura soil types (Table 1.16). Most species identified were isolated at both sites, with only five more species being present on clover and ryegrass roots growing in Te Kowhai soils. However, there were some differences in the prevalence of some fungi between the two soil types. *Cylindrocarpon destructans, Fusarium* spp. and *Idriella bolleyi* were more frequently isolated from plants growing in Horotui soil. *Aspergillus* spp., *Codinaea fertilis*, *Cylindrocladium scoparium* and *Trichoderma* spp. were more frequently isolated from plants in Te Kowhai soil. These differences were very noticeable, *C. fertilis* for example, comprised almost 20% of fungi at the Te Kowhai plots, but only 5% at the Horotui plots (Table 1.16) and this pattern was repeated for *Fusarium* spp. which were 26% of fungi at Horotui but only 14% at Te Kowhai. *Ramichloridium scultzeri* was notably more common in both the Ruakura soils, but was rarely recorded from roots at Whatawhata.

Table 1.16 Total number and percentage of fungal species isolated from surface sterilised root segments sampled from the two Ruakura soil types.

Ruakura soil type	HOROTUI sandy loam		TE KOWHAI silt loam	
FUNGAL SPECIES	Count	%	Count	%
Hyphomycetes				
-*Acremonium* spp.	33	2.2	12	*
-*Aspergillus* spp.	3	*	18	1.3
-*Codinaea fertilis*	75	5.0	253	18.5
-*Cylindrocarpon destructans*	62	4.1	29	2.1
-*Cylindrocladium scoparium*	15	1.0	45	3.3
-*Fusarium* spp.	398	26.5	195	14.2
-*Gliocladium* spp.	30	2.0	29	2.1
-*Idriella bolleyi*	17	1.1	8	*
-*Paecilomyces* spp.	14	*	22	1.6
-*Penicillium* spp.	13	*	22	1.6
-*Periconia macrospinosa*	18	1.2	9	*
-*Ramichloridium schultzeri*	18	1.2	6	*
-*Trichoderma* spp.	47	3.1	130	9.5
-*Verticillium* spp.	25	1.7	14	1.0
Pycnidial fungi	**35**	**2.3**	**33**	**2.4**
Sterile Fungi	**509**	**33.8**	**405**	**30.0**
Yeasts and allied species	**16**	**1.1**	**9**	*
Zygomycetes	**60**	**4.0**	**47**	**3.4**
Bimuria novae zelandiae	41	2.7	21	1.5
Other Fungi	76	5.1	64	4.7
TOTAL	**1505**	**100**	**1371**	**100**
Total no. of species isolated	**69**		**74**	

* Fungal isolation frequency <1%

Appendix 2 has the complete list of fungal species isolated from both soil types.

III. SURVEY THREE

A total of 333 fungi were obtained from 320 root segments (Table 1.17), with no fungi isolated from the remaining 80 segments. The percentage of root segments colonised by fungi ranged between 91% colonisation of subterranean clover segments to 64% colonisation of soft brome segments (Table 1.17). The lower number of soft brome segments colonised by fungi was the main reason for survey three having a low percentage fungal colonisation of roots compared to the species sampled in surveys one and two. No bacteria were isolated in this survey.

Table 1.17 Total number of root segments in survey one that were colonised by fungi, bacteria or remained uncolonised.

	Yorkshire fog	Lotus	Sub. clover	Soft brome	Total
No. of segments plated	100	100	100	100	**400**
No. of fungi isolated	88	82	97	66	**333**
No. of segments colonised by fungi	83	81	92	64	**320**
% root colonisation by fungi	83%	81%	92%	64%	**80%**
No. of bacteria isolated	0	0	0	0	**0**
No. of uncolonised segments	17	19	8	36	**80**

The root mycoflora isolated from these four plant species (Table 1.18) was similar to the root mycofloras of ryegrass, browntop, sweet vernal and white clover (Table 1.10). A large number of species (45), from 24 genera, were isolated in this survey, and there were differences between the number of species isolated from each plant species. Subterranean clover had a higher diversity of root colonising species (25 species) than lotus and Yorkshire fog which had a lower species diversity (18 species). The lower fungal colonisation of soft brome roots did not lower the diversity of species isolated from this host, as 20 species were obtained from root segments.

Although the sample size was smaller in this survey, seven fungi not isolated in either survey one or two were isolated from these four pasture species. These were; *Tricellula* sp., *Verticillium lecanii*, both from subterranean clover roots (Table 1.18), and five additional penicillia; *P. expansum, P. dendriticum, P. oxalicum, P. rastrickii* and *P. rugulosum* (Appendix 2). *Codinaea fertilis* was the most commonly isolated fungus, and was frequently found on lotus and subterranean clover roots, although it was absent from soft brome roots. *Fusarium* was the most commonly isolated genus and *F. oxysporum* was the second most isolated species. *Periconia macrospinosa* was also frequently isolated from all plant species, but particularly from the roots of lotus (17.1%) and Yorkshire fog (20.5%). Other common genera were; *Penicillium, Paecilomyces, Aspergillus, Trichoderma* and *Mortierella,* all of which were common in surveys one and two. Sterile fungi comprised 12.6% of isolates found in this survey which was a lower occurrence than in both survey one and two, where they comprised 40% of isolates. Other root fungi, such as *Periconia macrospinosa, Codinaea fertilis, Idriella bolleyi* and fusaria, were isolated more frequently on lotus and subterranean clover roots and this may explain the lower colonisation by dark sterile fungi. The results of this survey also contrasted from the previous two surveys in that *Gliocladium roseum* was isolated with a very low frequency and that *Acremonium* species were absent.

These isolation results indicate that most fungi isolated were not host specific and colonised a wide number of pasture plant hosts. However, higher numbers of *Codinaea fertilis* on lotus and subterranean clover indicate this species was more prevalent on leguminous pasture hosts. *Tricellula* sp. and *Verticillium lecanii* were only isolated from subterranean clover, but a larger survey is needed to distinguish host specificity from an aggregated, localised patch of root colonisation.

Table 1.18 Total number and percentage of fungal species isolated from surface sterilised root segments of Yorkshire fog, lotus, subterranean clover and soft brome.

Pasture plant	Yorkshire fog	Lotus	Subterranean clover	Soft brome		
Fungi species:					**Total**	**Total%**
Aspergillus spp.	4	1	1	1	**7**	1.8
Codinaea fertilis	8	20	23	-	**51**	15.3
Dactylaria acerosa	1	1	-	2	**4**	1.2
Fusarium spp.	18	25	15	8	**66**	19.8
-Fusarium oxysporum	14	19	7	6	**46**	13.8
Gliocladium roseum	-	3	1	1	**5**	1.5
Mariannaea elegans	-	-	3	4	**7**	2.1
Idriella bolleyi	1	1	7	-	**9**	2.7
Paecilomyces spp.	4	2	-	1	**7**	0.9
Penicillium spp.	2	2	6	11	**21**	1.5
Periconia macrospinosa	18	14	9	5	**46**	13.8
Trichoderma spp.	8	2	7	13	**30**	3.3
Tricellula sp.	-	-	6	-	**6**	1.8
Verticillium lecanii	-	-	3	-	**3**	0.9
Coelomycetes	5	2	-	1	**8**	2.1
Sterile dark fungi	10	6	3	5	**24**	7.2
Sterile hyaline fungi	5	-	7	6	**18**	5.4
Zygomycetes	2	1	5	5	**13**	0.9
Other fungi	2	2	2	3	**9**	0.3
TOTAL	**88**	**82**	**97**	**66**	**333**	
Total no. Species isolated	**17**	**17**	**25**	**20**	**45**	

Appendix 2 contains the complete isolation frequency of all fungal species.

Few differences were observed in the root mycofloras of Yorkshire fog, lotus and subterranean clover where many fungi had similar isolation rankings (Table 1.19), *Periconia macrospinosa, Codinaea fertilis, Fusarium oxysporum* and sterile fungi were all ranked in the top four fungi isolated. In comparison, soft brome had a different isolation ranking with *Trichoderma hamatum, Penicillium chrysogenum* and *Mariannaea elegans* highly ranked, and notably, *Codinaea fertilis* not isolated from this host plant. Root colonisation of soft brome also differed in that highly ranked fungi had lower total isolation percentages. This indicates the fungal population in soft brome roots had a high species diversity with low isolation frequency rather than a few dominant species with high isolation frequencies. The root mycoflora of soft brome was, however, similar to the other plant species in that sterile fungi and *F. oxysporum* were ubiquitous on its roots.

Table 1.19 The rank, number and percentage isolation of the seven most common fungi obtained from pasture plants sampled in survey three.

Yorkshire Fog				Subterranean clover			
	Rank	No.	%		Rank	No.	%
Periconia macrospinosa	1	18	20.5	*Codinaea fertilis*	1	23	23.7
Fusarium oxysporum	2	14	15.9	*Periconia macrospinosa*	2	9	9.3
Sterile dark fungi	3	10	11.3	*Fusarium oxysporum*	3	7	7.2
Codinaea fertilis	4	8	9.1	*Idriella bolleyi*	3	7	7.2
Pycnidial fungi	5	5	5.7	Sterile hyaline fungi	3	7	7.2
Sterile hyaline fungi	5	5	5.7	*Tricellula* sp.	4	6	6.2
Aspergillus niger	6	4	4.6	*Fusarium avenaceum*	5	5	5.2
Trichoderma harzianum	6	4	4.6				

Lotus				Soft brome			
	Rank	No.	%		Rank	No.	%
Codinaea fertilis	1	20	24.4	*Trichoderma hamatum*	1	7	10.6
Fusarium oxysporum	2	19	23.2	*Fusarium oxysporum*	2	6	9.1
Periconia macrospinosa	3	14	17.1	Sterile hyaline fungi	2	6	9.1
Sterile dark fungi	4	6	7.3	*Penicillium chrysogenum*	3	5	7.6
Fusarium culmorum	5	5	6.1	*Periconia macrospinosa*	3	5	7.6
Gliocladium roseum	6	3	3.7	Sterile dark fungi	3	5	7.6
Paecilomyces lilacinus	7	2	2.4	*Mariannaea elegans*	4	4	6.1

Survey three was undertaken in March, when the soil moisture content was lower and soil temperatures higher, particularly plots on northern slopes (Table1.1), than the those measured in November, when the first two surveys were undertaken. Although the same dominant fungi were isolated in survey three, and the host plants were different, there were notable differences in the isolation frequency of some fungi. The higher occurrence of *C. fertilis, P. macrospinosa* and *Fusarium* spp. (Tables 1.18 & 1.19) may be due to a change in the soil environment rather than the different host species sampled. The sample size of root segments plated was considerably lower than those of the first two surveys, but was large enough to indicate the frequency of fungi that were present in roots.

1.4 DISCUSSION

The fungal communities within Waikato pasture plant roots were characterised by a few frequently isolated species and a large number of infrequently isolated species. All dominant species were distributed on all host plants at both study sites. The less frequently isolated fungi were aggregated in their distribution often appearing as localised patches of colonisation, on one or two hosts at one site. Root-colonising fungi have long been reported to have scattered and patchy distributions in the soil (Garrett 1950) which is a result similar to that obtained here for species such as *Thielaviopsis basicola* and *Cylindrocladium scoparium*. Species of fungi isolated in this study invade plant roots either as damaging parasites, or pioneer and secondary colonists which help decompose dying tissue and the recycling of plant nutrients (Appendix 3).

The apparent fungal composition of roots observed isolated in this study was biased towards fungi which are able to grow on WA media from surface sterilised roots at 20°C. Isolations from root zones and soils will always reflect the bias imposed by the isolation method (Warcup 1950, Kreutzer 1972), and this has been a constant constraint when researching root fungi. Some important root and rhizosphere fungi, such as the oomycetes, were therefore excluded as they generally do not survive rigorous surface sterilisation with sodium hypochlorite, and some oomycete species can only be isolated using specific baiting techniques (Martin 1992, Mitchell and Kannwischer-Mitchell 1992). The ecologically important Zygomycetous Endogonales (vesicular-arbuscular mycorrhizal fungi), cannot be isolated into culture and must be quantified by direct examination methods (Gams 1992b, Moutoglis *et al.* 1995). Mycorrhizal and Oomycetous fungi which were not obtained in this survey, have been reported to be significant components of pasture root mycofloras (Powell 1976, Skipp and Christensen 1981,1983, Abad *et al.* 1994). Basidiomycetes with clamp connections were uncommon in this study, comprising less than 1% of isolates, which is a result similar to that reported by Waid (1957) and Thornton (1965). This class of fungi is common in pasture soils (Warcup 1959) and different methods are also required to isolate these fungi (Taylor 1971). Basidiomycetes rarely sporulate in culture (Warcup 1959, Warcup and Talbot 1962) and therefore many isolates could have been classed as sterile fungi in the results. Separate surveys with specific isolation techniques could be undertaken in the future to determine the presence of oomycetes, basidiomycetes and Mycorrhizal fungi in Waikato pastures.

Although this study aimed to isolate all important root-colonising fungi into culture, it was not possible to isolate every component of the mycoflora given the constraints of time. Cultural methods usually have a positive bias for isolation of fungi, such as *Trichoderma* and zygomycetes, which have rapid colony growth (Warcup 1959, Bell 1995, pers comm.). However, the rapid removal of these fungi from the WA isolation plates enabled slow growing fungi, such as *Trichosporon* and *Dactylaria* to be recovered in each survey and showed that slow growing fungi were an important component of the pasture root mycoflora. A random selection of living roots and segments plated in this study enabled an unbiased mixture of both old and young roots to be sampled. This was important as fungal populations have been reported to differ depending on the age of roots. Young roots are either uncolonised (353 segments in this study) or have high populations of fusaria, penicillia, oomycetes and zygomycetes, while older roots are successively more colonised by sterile fungi, *Gliocladium roseum* and *Fusarium oxysporum* (Waid 1957 1974, Parkinson *et al.*1963, Dix 1964, Gadgil 1965, Taylor and Parkinson 1965). The incubation temperature may influence the isolation frequency of some fungi (Carreiro and Koske 1992), and very different microfungi can be obtained from the same sample with different isolation temperatures. For example *Fusarium nivale* is common on Gramineae plant roots, but most frequently at temperatures between 5-10°C (Colhoun 1979), and therefore this survey is biased towards the isolation of mesophilic root-fungi.

Most of the fungi identified have been previously described as widespread soil genera (Barron 1968, Domsch *et al.* 1980, Gams 1992), and in particular as members of common soil hyphomycete genera. As most mitosporic fungi have not been correlated with their meiotic teleomorphs in the ascomycetes and basidiomycetes, the hyphomycetes is still the largest class of described fungi (Hawksworth *et al.* 1995) and is probably one of the reasons why they were the largest numerical component of the pasture root mycoflora. Many root-colonising hyphomycetes recorded in this study, such as *Fusarium* and *Trichoderma,* also live saprophytically in soil (Bywater 1959, Mangenot and Diem 1979, Domsch *et al.* 1980) and therefore readily invade roots as they grow through the soil. Many hyphomycetes also produce copious asexual conidia to survive in the soil which readily germinate and invade roots (Burgess *et al.* 1988, Allan *et al.* 1992, Hawksworth *et al.* 1995).

Root-colonising fungi in pasture are not host specific and the majority of species were isolated from more than one plant host. A notable exception was *Bimuria novae zelandiae* which was exclusively and frequently identified from clover. *Codinaea fertilis, Cylindrocladium scoparium* and several *Fusarium* species were found on all host plants but were more prevalent on clover or leguminous plant roots, indicating clover was more susceptible to invasion by these fungi. There were no fungi which were consistently host specific to grass hosts although *Trichoderma polysporum* was most frequently isolated from perennial ryegrass at both Ruakura and Whatawhata which may indicate host preference.

There were few differences between the species of fungi isolated from hill country pasture plants at Whatawhata and those isolated from dairy pastures at Ruakura. *Dactylaria acerosa, Thozetella tocklaiensis* and sterile hyaline fungi group 3 were the only fungi that were prevalent on plant roots at Whatawhata but were absent or rare at Ruakura. Despite the difference in botanical composition and soil types of the two pasture sites, the same diversity of fungal species was consistently obtained. The difference in botanical composition between north and south facing pastures at Whatawhata did not affect the host specificity of most fungi isolated. This result again indicates that these fungi invade roots encountered in the soil, regardless of the host species. This was further demonstrated as most of these fungi have a worldwide distribution, and have been reported from many different plant hosts (Appendix 3) and are not generally host specific to pasture plants.

Soil type also appeared to have little effect on root-colonising fungi. The same characteristic mycoflora was obtained from all plants sampled from plots within the three soil types. This was particularly shown at the Ruakura plots where the fungi obtained from roots were similar for two different soil types. There were, however, observable differences in the isolation frequencies between some fungi in these two Ruakura soil types. This could have been influenced by microbial interactions on the roots or localised

differences in spatial distribution of fungal species as much as soil type. Soil type has previously been demonstrated to have little influence on the fungi living in roots as other surveys of roots in pastures throughout New Zealand have shown few differences between the fungi obtained (Thornton 1965, Skipp and Christensen 1983, 1989a). Environmental factors such as soil temperature, moisture and pH can be important in affecting root-fungi populations (Gams 1992, Dix and Webster 1995). For example *Codinaea fertilis* has been isolated from roots growing in many soil types but only from warm areas of New Zealand (Menzies 1973a, Skipp and Christensen 1983, 1989a), and the isolation frequency of this species from clover roots increases in summer with warmer temperatures (Burch pers. comm.). This result could also be interpreted in the present study, where *C. fertilis* had a higher proportional isolation frequency from warm, dry, north- facing pasture slopes in survey one, and in survey three when soil temperatures were higher with low soil moisture. The different relative isolation frequencies of the dominant fungi recorded in this later survey could be due to the combination of environmental factors or the different hosts surveyed.

Fungal communities in soil and roots usually show some seasonal variation (Bissett and Parkinson 1979a) and therefore sampling from pasture roots over several seasons is an important area for further research. Roots in this study were only sampled from the A soil horizon (mainly so that plant host for each root could be determined) however, it has been reported that fungal communities can change with soil horizon depth (Bissett and Parkinson 1979b, Dix and Webster 1995) and this could also be an area for future research within Waikato pastures.

Fungal abundance on grass roots has been shown experimentally to be influenced by the nutrient status of the surrounding soils (Turner and Newman 1984) where roots growing in nutrient poor soils have a higher abundance of fungi living in and on the roots than roots growing in high fertility soils. There was little evidence of this in the present study as roots from the low fertility pasture plots at Whatawhata were colonised with the same frequency as roots in the high fertility plots at Ruakura. However, *Fusarium culmorum* was notably more abundant on plants sampled from high fertility Ruakura plots. This result is similar to those previously reported by Kreutzer (1972), who isolated fusaria from soils and roots of low and high fertility grassland in Colorado and found *F. culmorum* to be the most frequently encountered fungus in high fertility pastures. As 78% of *F. culmorum* isolates were from roots at Ruakura, the soil fertility status may be an explanation for this result, but further investigation is required.

The results of this study are similar to those reported in other previous surveys of pasture roots in New Zealand. These surveys undertaken by Thornton (1965), Skipp and Christensen (1983 1989a) and (Bonish 1989) have all demonstrated that pasture roots contain a unique mycoflora of root fungi comprising a mixture of common soil and rhizosphere saprophytic fungi which have the ability to invade root tissue, and a small

component of less common non saprophytic fungi which are specifically isolated from roots. Despite differences in isolation methods between previous surveys and this study, a unique internal root mycoflora has been consistently isolated from a range of soils, sites and plant hosts from pastures throughout New Zealand and these commonly reported fungi are summarised in Table 1.20.

Sterile isolates were the most abundant fungi isolated from roots in New Zealand pastures comprising up to 55% of the mycoflora (Table 1.20). These fungi are therefore important but little is known on their function and diversity which is because of the difficulty in working with undefined taxa. These surveys were also similar in that *Trichoderma,* sterile fungi and zygomycetes were more frequently isolated from ryegrass while *Codinaea* and *Fusarium* species were more frequently found on clover roots (Table 1.20). Skipp and Christensen (1989a) additionally reported that *Phialophora radicicola* was host specific to ryegrass, but this species appears to be absent or uncommon at the Waikato pasture sites surveyed by Bonish and those in the present study. *Phialophora radicicola* has also been reported to be more frequently observed in ryegrass roots by direct observation methods, but is more rarely observed using cultural methods (Labruyere 1979). In contrast, the genus *Paecilomyces* was common in the Waikato surveys but was absent or rare elsewhere. Other differences between the root mycofloras of clover and ryegrass in these surveys were minimal, although, *Bimuria novae zelandiae* and *Chrysosporium* were host-specific to white clover. *Bimuria novae zelandiae* is an indigenous ascomycete found only in New Zealand and has to date only been reported from soil and clover roots (Table 1.20, Appendix 3). However, the root mycoflora of indigenous flora in New Zealand has scarcely been investigated and this fungus may also be common on indigenous hosts.

A distinctive yeast flora was isolated from roots in this study. The two root-colonising yeasts, *Geotrichum candidum* and *Trichosporon cutaneum,* are both mycelial yeast species, as is the closely related yeast-like species *Aureobasidium pullulans.* This result contrasts to the usual reported isolation of unicellular *Cryptococcus* and *Candida* yeast species, which are dominant in New Zealand pasture soils (di Menna 1960).

Penicillium and *Acremonium* species were also characteristic components of the pasture root mycoflora. The identification to species of these isolates in the present study, has elucidated which species are important and most frequently isolated. Although 19 species of penicillia were recorded, only four species; *P. brevicompactum, P. chrysogenum, P. janthinellum* and *P. simplicissimum*, were consistently isolated from both sites (Appendix 2,3). This genus was more frequently isolated from Whatawhata plant roots where plots had a higher soil moisture content. Dix and Webster (1995) reported penicillia were more common from soils with low soil moisture which contrasts to the present results, however further research including soil isolations, would have to be undertaken to confirm soil moisture is not a factor for this result. Six species of *Acremonium* were recorded, and this study indicate *A. curvulum* and *A. kiliense* as being

the numerically important species living in roots. *Gliocladium, Periconia* and *Cylindrocarpon* were also frequently isolated in all surveys, and combined with the other groups listed in Table 1.20, provide a measure of the expected diversity of dominant fungi that colonise roots of New Zealand pasture plants.

Table 1.20 The internal root mycoflora reported from surveys of New Zealand pasture plants (% frequency isolation from surface sterilised root segments).

FUNGI	Thornton[1]		Skipp & Christensen[2]		Bonish[3]		Waipara[4]		
	Rye	Clover	Rye	Clover	Rye	Clover	Rye	Clover	Total*
Acremonium	-	-	<1	1.8	1.3	-	1.6	1.9	1.7
Bimuria	-	-	-	16.2	-	3.0	-	3.5	1.2
Codinaea	-	2.5	9.1	7.7	12.6	12.6	6.9	15.8	9.8
Chrysosporium	-	-	-	3.4	-	6.3	<1	<1	<1
Cylindrocarpon	8.2	13.2	1.7	2.3	10.0	5.0	2.5	3.0	1.9
Fusarium	12.7	19.2	8.8	7.5	11.0	50.0	14.8	23.4	16.1
Gliocladium	6.0	6.2	1.2	2.4	1.0	3.4	2.5	2.7	2.3
Paecilomyces	-	-	-	-	-	1.4	1.8	1.9	1.6
Phialophora	1.2	<1	17.8	-	-	-	-	-	-
Penicillium	4.0	4.5	2.5	1.0	-	1.0	2.9	1.4	3.0
Periconia	1.5	<1	3.7	<1	11.7	3.0	1.0	<1	1.7
Pycnidial fungi	<1	2.0	1.1	7.2	-	-	1.8	2.1	1.9
Sterile fungi	55.0	44.0	32.0	18.9	27.0	6.3	43.0	22.9	39.0
Trichoderma	1.3	1.0	3.1	1.0	15.0	1.7	7.0	4.4	5.2
Zygomycetes	14.0	6.0	1.9	<1	1.3	-	3.3	2.6	2.7
Total fungi	1156	611	3348	7520	223	175	2559	2298	7232

[1] Thornton (1965), results are for both plants is the mean % frequency of fungi from 6 soil types.
[2] Clover results from Skipp & Christensen (1983), ryegrass from Skipp & Christensen (1989a).
[3] Bonish unpublished results from a survey of Ruakura pastures undertaken in 1990.
[4] Results from the present study, * = Overall % isolation frequency of fungi from all three surveys from 8 host plants as shown in Table 1.6 and Appendix 2.

The diversity of fungi isolated from New Zealand pasture is also similar to that reported from some overseas studies on pasture root fungi (Waid 1957,1974, Nicolson 1959, Gadgil 1965, Labruyere 1979) where this characteristic mixture of fungal species shown in Table 1.20 has again been reported. Chloramphenicol tolerant bacterial species were isolated from root segments despite the WA isolation media being amended with this antibiotic, indicating antibiotic resistance. *Pseudomonas corrugata* was the commonest bacterium obtained, and this species is antagonistic to many fungi (Ryder and Rovira 1993, Pankhurst and Lynch 1995). This could be the reason why root segments colonised by these bacteria were devoid of fungal mycelium.

In summary, the fungi isolated from pasture plant roots were dominated by ten hyphomycete genera, sterile fungi, pycnidial fungi and zygomycetes. Therefore root-colonising fungi, although forming a natural ecological group, are diverse in their systematic affinities. These species were not only ubiquitous on all plants at both sites but have also been reported from clover and ryegrass pastures throughout New Zealand. The

role and significance of these fungi on plant germination and growth of pasture plants will be assessed as the third part of this study.

CHAPTER TWO - MORPHOLOGY AND CHARACTERISATION OF ROOT-COLONISING STERILE FUNGI ISOLATED FROM WAIKATO PASTURES.

"A science can scarcely be said to exist before its material, whether it be species or elements, has been organised and classified"
Wertenbaker 1974

2.1 INTRODUCTION

Sterile fungi (or *Mycelia sterilia*) are anamorphic mycelia which do not produce true conidia (Hawksworth *et al.* 1995). They are an artificial assemblage of sterile asexual mycelia which are classed as Deuteromycetes (Fungi imperfecti). Although conidia are absent, non-dehiscent propagules (such as allocysts, chlamydospores, bulbils and sclerotia) and similar structures (such as monilioid cells or hyphal swellings) may be produced in some genera. Sterile fungi may be states of ascomycetes, basidiomycetes or other mitosporic fungi.

Classification of sterile fungi has to date been attempted by four authors. (Carmichael *et al.* 1980) divided sterile fungi into three types, those with bulbils (13 genera), those with sclerotia (3 genera) and those with only hyphae (5 genera). Domsch *et al.* (1980), listed four illustrated genera and von Arx (1981) listed 11 genera of sterile fungi but only provided illustrations of one bulbil-producing genus. The most recent classification is the 28 genera comprising 200 species of sterile fungi that have been officially described and classed as agonomycetes by Hawksworth *et al.* (1995). Although the order Agonomycetales includes well defined genera, such as *Papulospora,* it is mostly a catchall repository for a large number of nondescript mycelial isolates (Barron 1968). Parmeter and Whitney (1970) contend that extending binomial nomenclature classification systems to include sterile fungi is untenable, since it would lead to the erection of species which would include mycelial states of basidiomycetes and ascomycetes. The identification of fungi from written descriptions of mycelia is extremely difficult due to the inherent simplicity of the vegetative system of most fungi (Parmeter 1965), and it is also difficult to describe a sterile mycelium with the assurance that another worker could subsequently identify that mycelium from its written description (Parmeter 1965, Parmeter and Whitney 1970). Therefore, it is also vital to provide illustrations when describing any sterile fungus (Hall 1987).

Although a large number of soil isolates lack reproductive and resting structures in culture, it cannot be assumed that these isolates are truly sterile, as many fungi fail to sporulate in culture under laboratory conditions (Leach 1962, Warcup and Talbot 1962, Hall 1987). A large number of ascomycetes and basidiomycetes which lack an asexual state will not produce a sexual phase in culture, and some hyphomycetes do not develop a

conidial state in culture. Such isolates will often be incorrectly classified as agonomycetes (Barron 1968). However, increased sporulation of fungi in culture can be achieved by the use of media supplemented with a variety of substrates and with media incorporating different single carbon and nitrogen sources or supplemented with various nutrients (Sloan *et al.* 1960, Hall 1987, Paterson and Bridge 1994, Smith and Onions 1994).

Sterile fungi have been reported to be isolated with high frequency from plant roots (Taylor and Parkinson 1964, Singh 1980, Chu-Chou and Grace 1982, Hall 1985, Hall 1987) and have been frequently reported from pasture plant roots (Waid 1957, 1974, Gadgil 1965, Davidson *et al.* 1977). In New Zealand, both hyaline and dematiaceous forms are commonly recorded on; perennial ryegrass (Thornton 1965, Skipp and Christensen 1981, 1989a, Falloon 1985, Sarathchandra *et al.* 1995), Italian ryegrass (Falloon 1985), white clover (Thornton 1965, Skipp and Christensen 1982, Skipp *et al.* 1982, Sarathchandra *et al.* 1995) and red clover (Skipp *et al.* 1986, Nan *et al.* 1991b). Sterile fungi are not only commonly isolated from pasture plant roots, but also from the surrounding pastoral soils (Thornton 1958, 1965, Morrison *et al.* 1959, Jackson 1965), and sterile dark isolates have been more frequently recorded from pasture roots and soils than hyaline isolates.

Sterile fungi which colonise plant root tissues exist as saprophytes (Taylor and Parkinson 1965, Thornton 1965, Pugh 1967) or parasitic necrotrophs which can cause soilborne diseases (Howard *et al.* 1977, Sumner *et al.* 1979, Green *et al.* 1983, Stegman de Gurfinkel *et al.* 1994). Sterile fungi have also been reported to be seedborne and soilborne pathogens which have a detrimental effect on seed germination (Cother 1977, Pitty *et al.* 1987). In contrast, sterile root-colonising fungi can also benefit plants by promoting their growth or by the suppression and antagonism of soilborne and root diseases (Dewan and Sivasithamparam 1989a, 1989c, Narita and Suzui 1991, Shivanna *et al.* 1994). A remarkable sterile red basidiomycete which colonises the roots and seeds of wheat and ryegrass plants is a biocontrol agent of the take-all fungus (*Gaeumannomyces graminis* v. Arx & Olivier var. *tritici* Walker) as it is suppressive of take-all root disease (Dewan and Sivasithamparam 1989c). This sterile fungus also promotes plant growth of ryegrass, wheat and other rotational crops by increasing root and shoot weights, and root length (Dewan and Sivasithamparam 1989b, 1990, 1991).

The occurrence of sterile mycelium was a noticeable and important characteristic of the mycoflora pasture roots in this study, as a total of 2824 sterile fungi were isolated. These were almost 40% of all fungi obtained and confirmed these non-sporing sterile forms to be important component of pasture root mycoflora. A total of 2216 or 78% of sterile isolates were dematiaceous, and the remaining 608 sterile isolates were hyaline. Sterile fungi were recorded on all pasture species at both sites of experimental sampling and were therefore regarded as typical of the Waikato pasture root mycoflora. There is some

discussion as to whether many fungi in culture are truly sterile (Hall 1985), as in culture may not provide the required conditions to trigger production of asexual and sexual structures. For the purposes of this study any fungi not sporulating on standard laboratory media in the presence of near-UV light were recorded as sterile (Leach 1962, Hall 1985).

A detailed study of sterile fungi which colonise roots is important as they have often been discarded by investigators and therefore their role has been understated because of a lack of information (Waid 1974, Hall 1986). As sterile fungi were a large component of the Waikato pasture root mycoflora further taxonomic examination of this unknown and amorphous assemblage of isolates was undertaken. The work outlined in this chapter investigates the cultural morphology of both dark and hyaline sterile isolates and a variety of methods were utilised to induce sporulation as well as separate the large number of amorphous isolates into taxonomic groups. Root colonisation and pathogenicity of these isolates was also investigated.

2.2 MATERIALS AND METHODS

2.2.1 MORPHOLOGICAL EXAMINATION OF STERILE ISOLATES

2.2.1 (a) Classification of sterile isolates using cultural characteristics.

Cultures isolated from plant roots which had not sporulated after four weeks growth on PCA at 20°C, or after six weeks on HA at room temperature in daylight were classed as sterile. Cultural growth rates and colony characteristics of pigmentation and hyphal branching patterns were also determined on both these media. Isolates were placed in groups of hyaline or darkly pigmented groups with similar hyphal growth rates. Some isolates were initially hyaline in culture but became progressively pigmented with age, and these were grouped as sterile dark forms. From this classification 15 broad groupings of isolates were identified.

Thirteen of these groupings had distinct growth rates or hyphal characteristics while the remaining two groups contained either hyaline or dark isolates which did not fit the parameters of the other groups and were therefore an amorphous grouping of varied isolates. These isolates were not studied further because of the number of isolates obtained. A sample of 40 isolates from each of the 15 groups were plated onto PDA and incubated at 20°C, after 21 days growth they were placed under near-UV light for 7 days.

Isolates were reassessed both on cultural morphology (growth rates and hyphal morphology) and appearance of hyphae and associated structures by light microscopy. This process allowed similar isolates to be again placed in the same 13 sterile groups (SG).

Twenty isolates from each SG were then plated in duplicate onto eleven media; malt extract agar (MEA), soil extract agar (SEA), hay agar (HA), potato carrot agar (PCA), vegetable juice agar (V8), Czapek dox agar (CPZ), Sabouraud agar (SAB), yeast extract agar (YEA), potato dextrose agar (PDA), sucrose asparagine agar (SUC) and oat agar (OA). All cultures were submitted to a 12 h near ultra violet (UV) (black light) irradiation /12 hr dark cycle for up to eight weeks at 20°C (Leach, 1962). After two weeks, growth on one Petri plate of each isolate was subjected to cold treatment by incubating at 2°C for 48 hr before being reincubated at 20°C under near-UV light (Leach 1962, Hall 1987).

Colour range of both the colony and reverse of the plate was determined using the Methuen handbook of Colour (Kornerup and Wanscher 1978). Film development and processing has affected the colour of some of the plates of these sterile groups.

2.2.1 (b) Nucleus and septum staining.

Nuclei and septa of fungal mycelia from each of the 12 sterile groups were observed by a rapid, clear staining technique using Hoechst Dye (Kangatharalingam and Ferguson 1982, Hua' an *et al.* 1991).

A stock solution of Hoechst Dye 33258 Calbiochem was added to 25 ml of SDW and heated to 37°C in a water bath until the dye was dissolved. Two buffer solutions were also prepared. A pH buffer of 7.8 was made using 0.1 M KH_2PO_4 and 0.1 M NaOH to observe nuclei and to observe septa, a pH buffer of 10.5 using 0.025 M H_3BO_3 and 0.1 M NaOH was made (Hua' an *et al.* 1991). Staining solutions were prepared by adding 0.6 ml of Hoechst stock dye to 50 ml of each buffer solutions.

Glass microscope slides were immersed in 96% ethanol, drained and flamed. Slides were immersed in and coated with PCA, then placed on glass rods to set in Petri dishes containing sterile filter paper moistened with SDW. A plug of mycelium from each sterile group was removed with a scalpel from the margin of a PCA culture and transferred to a glass microscope slide. Petri dishes were incubated at 20°C in darkness for 48 hr to one week depending on the growth rate of each group. Twenty fungal isolates from each sterile group were tested both for nuclei number and type of septa.

After incubation, slide cultures were placed in a laminar flow cabinet to dry the agar coating. A drop of each staining solution was added to each half of the colony which allowed both stains to be made twice on one slide. Two coverslips were placed over both drops on each slide and were observed under a fluorescence microscope.

2.2.1 (c) Effect of temperature and pH on growth rates on PCA.

The colony diameter of each sterile group was determined at six pH levels. The pH of PCA was adjusted using HCl or NaOH to 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. A 5 mm plug of fungal mycelium of ten isolates from each group was inoculated onto the centre of PCA plates. Plates were incubated in the dark at 20°C and the diameter measured after 14 days .

The cultural growth rate of each sterile group was also determined at six temperatures. A 5 mm plug of fungal mycelium of ten isolates from each group was inoculated onto the center of PCA plates. Plates were incubated in the dark at 10, 15, 20, 25, 30, 35°C and colony diameter measured after 14 days.

2.2.1 (d) Effect of fungicide on growth rates on PCA.

The ability of benomyl to affect the colony growth of eight sterile groups as well as *Thozetella tocklaiensis, Fusarium crookwellense* and *Cylindrocladium scoparium* was tested. Benomyl (1-butyl-carbamoyl 2-benzimidazole, Bayer Ltd) was dissolved in ethanol and added in measured amounts to molten PCA to give plates with six levels of fungicide concentration 0.1, 0.25, 0.5, 1.0 and 5.0 mg a.i./ml. A 5 mm plug of mycelium from five isolates from each group was inoculated onto the plates and incubated in the dark at 20°C. Colony diameters were measured after 14 days growth.

2.2.1 (e) Assimilation of carbon and nitrogen sources.

The ability of sterile fungi to utilise particular compounds as their sole source of carbon or nitrogen was tested. Assimilation was determined by measuring the radial colony growth of each isolate after being inoculated onto assimilation plates with either 15 carbon compounds or 16 nitrogen compounds. Growth of five isolates from eight sterile groups as well as *Thozetella tocklaiensis* was assessed on a chemically defined basal medium with either a single carbon or nitrogen source (Paterson and Bridge 1994).

The 15 carbon sources (Table 2.1) used in this study were added to a minimal salts NH_4Cl source medium (MSM, Appendix 1) at a final concentration of 0.2% (w/v) with the pH of the media adjusted to 5.5. The 16 nitrogen sources (8 organic; 8 inorganic, Table 2.1) were added to the same minimal salts glucose medium (MSM, Appendix 1) also at a final concentration of 0.2% (w/v) with the pH of the media being adjusted to 5.5.

The assimilation medium plates were inoculated with a 5 mm plug of PCA culture mycelium and incubated in the dark at 20°C. Growth was measured after 7-14 days incubation.

Table 2.1 Carbon and nitrogen compounds tested for assimilation by sterile fungi.

Carbon sources: (15)	Nitrogen sources: (16)
	Organic:
Arabinose	Arginine
Cellobiose	Asparaginine
Fructose	Cysteine
Galactose	Glutamine
Glucose	Histidine
Inositol	Leucine
Lactose	Methionine
Maltose	Trytophan
Mannitol	Inorganic:
Mannose	Ammonium chloride [NH_4Cl]
Melizitose	Ammonium nitrate [$NH_4(NO_3)$]
Raffinose	Ammonium sulphate [NH_4SO_4]
Succinic acid	Potassium nitrate [KNO_3]
Trehalose	Potassium nitrite [KNO_2]
Xylose	Sodium nitrate [$NaNO_3$]
	Sodium nitrite [$NaNO_2$]
	Urea [H_2NCONH_2]

2.2.1 (g) Inoculation of sterile fungi onto axenically grown seedlings.

The rapid *in vitro* screening technique given in the methods of Chapter three, was used to determine pathogenicity and root colonisation of each sterile group on axenically grown seedlings of grasses and legumes. This technique has also been reported to induce sporulation of fungal species (Christensen *et al.* 1988) and was also used to induce sterile isolates to produce diagnostic structures. Seeds of eight grasses (perennial ryegrass, cocksfoot, sweet vernal, browntop, Yorkshire fog, soft brome, tall fescue, timothy) and four legumes (white clover, red clover, subterranean clover, lotus) were surface sterilised in 1% sodium hypochlorite solution for 5 minutes followed by three rinses in sterile water. Seeds were germinated on 1.5% WA in Petri plates and incubated at 20°C. Five surface sterilised seeds were placed in a line across each WA plate. Plates were aligned horizontally so that seeds would germinate vertically across the plates. After seven days, seedlings were inoculated with 5 mm PCA culture plugs of fungus and 10 days after inoculation were scored for root disease symptoms.

A disease score 0-5 was assigned to each seedling based on observable disease symptoms. The disease scores used were; 0 = white turgid roots with no visible symptoms; 1 = light discoloration (yellowing or browning) of root tissue; 2 = dark brown discoloration of root tissue and inhibition of root growth; 3 = root surface lesions present and inhibition of root growth; 4 = systemic root lesioning and necrosis; 5 = complete seedling death. A mean disease score was then calculated for each host by averaging all five disease scores for each host plant. A root segment from each inoculated seedling was

plated onto WA to reisolate the fungus from diseased tissue. A pathogenicity rating was calculated for each isolate on each host by averaging the five disease scores from plates for each treatment.

Hyphal penetration of colonised seedlings was determined microscopically. Twenty longitudinal and transverse sectioned roots were cleared in 10% KOH at 60°C, rinsed in 10% H_2SO_4 and stained with 5% trypan blue in lactophenol at 60°C. Root sections were then mounted on a slide in a drop of lactophenol and examined by light microscopy. Roots were rated on the extent of fungal hyphal colonisation. The colonisation scores used were; 0 = hyphal penetration absent; 1 = epidermal colonisation; 2 = cortex colonisation; 3 = inner cortex and vascular tissue colonisation.

2.2.1 (g) Growth of sterile fungi on different substrates.

Eight isolates representative from each sterile group were inoculated onto three substrates to induce sporulation and to determine the best production method to produce mycelium inocula for further experiments.

- Liquid culture V8 broth medium (VBM)

Each fungus was inoculated into 250 ml Erlenmeyer flasks, which contained 100 ml of autoclaved VBM (appendix 1). The flasks were incubated on a rotary shaker (120 rpm) for 21 days at 20°C.

- Kibbled wheat and perennial ryegrass seed

For the preparation of inocula, 1.1 kg of kibbled wheat was soaked in 1 l of distilled water, washed and autoclaved twice in an autoclavable bag at 120°C for 30 minutes. Sterilised wheat was dispensed in 50g lots into sterile 250 ml Erlenmeyer flasks which were each inoculated with 10 mycelial plugs (5 mm) obtained from the margins of each sterile group cultured on PCA for 7-14 days. Inoculated wheat was incubated at 20°C and shaken occasionally until all grains had been colonised by mycelium. The same method was used for the perennial ryegrass seed.

2.2.2 EFFECT OF STERILE FUNGI ON THE YIELD OF SHOOT AND ROOTS OF PASTURE PLANTS.

A mixture of kibbled wheat and liquid culture inocula were used to inoculate six common pasture plants to asses the influence of sterile fungi on plant shoot and root weight. Six plant cultivars, white clover (Huia), perennial ryegrass (endophyte-free Nui),

perennial ryegrass (*Acremonium lolii*-infected Nui), browntop (Egmont), sweet vernal (BZ2330) and soft brome (Whatawhata WT), were grown from surface sterilised seed in plastic pots (73 mm x 45 mm). Pots contained a mixture (10% w/w) of the kibbled wheat inoculum (7g) of each fungus and 70g of fumigated Horotui sandy loam soil. Five plants grown from surface sterilised seed were planted in each pot. Control plants were grown in soil mixed with sterilised kibbled wheat. The method of soil fumigation is given in the methods section of Chapter three.

A treatment of 1 ml of $1x10^9$ cells of *Rhizobium leguminosarum* biovar *trifolii* Dangeard (Strain 2163-89 from the international collection of micro-organisms from plants, PDD Herbarium, Landcare Research, Auckland, New Zealand), was added to all pots, one week after seed germination. This treatment was to ensure nodulation of clover for the trial, but was added to all host plants to ensure uniform treatment for each pot.

After three weeks growth, plants were again inoculated with a homogenised suspension of mycelium of each fungus grown in liquid culture. These mycelial inocula were prepared by pouring off the VBM broth from each liquid culture and washing the mycelium in distilled water. The mycelium was then homogenised in a Waring blender for 1 min and diluted with distilled water to obtain a suspension with 10^6 hyphal fragments /ml. Test plants were inoculated by adding 15 ml of mycelial suspension into each pot. The suspension was pipetted into the soil up to a 30 mm depth (measured with a marked pipette tip. Control plants were inoculated with 15 ml of sterilised (autoclaved for 20 min at 120°C) mycelial suspensions.

Plants were maintained for 5 weeks under artificial illumination (110 μ Einsteins m^{-2} sec^{-1}) in a controlled environment room. Temperature was kept at 20°C with 16h light and 8h dark periods. Plants were watered by weight periodically to maintain the soil at 70% of the soils water holding capacity. Pots were replicated 3 times per treatment and were randomly arranged in the growth room. Each pot was placed in a second outer pot to prevent cross contamination of spore suspensions through watering.

Plants were harvested five weeks after inoculation. Roots were washed and visually assessed for percentage root area that was affected by root rot. Each plant was assigned a root disease score 0-5 as follows: 0= Lesions and necrosis absent from roots; 1= 1-20% of total root area necrotic; 2= 21-40% of total root area necrotic; 3= 41-60% of total root area necrotic; 4= 61- 80% of total root area necrotic; 5= 81-100% of total root area necrotic.

Ten surface sterilized root segments from each plant of each species were plated onto WA to reisolate each of the inoculated fungi. All remaining roots were detached from shoot

material, then root and shoot components were placed in separate paper bags, and dried in a 60°C oven for 48 hours to determine dry weight yields.

2.2.3 ASSESSMENT OF ISOZYME ANALYSIS METHODS TO DISTINGUISH STERILE FUNGAL GROUPS

Isozyme analysis is a powerful biochemical technique that is being used routinely to characterise and identify fungal cultures (Bonde *et al.* 1993) and has also proved a useful method to investigate relationships within and among populations of fungi (Cruickshank and Pitt 1987, Goodwin *et al.* 1993, Christensen 1995). Isozymes are the molecular forms of an enzyme which usually have similar enzymatic properties but different amino acid sequences (Bonde *et al.* 1993). These differences can be differentiated by electrophoresis, which is used to separate isozymes on either starch or polyacrylimide gels. Following electrophoresis, isozymes are detected through the use of specific activity stains. Detection is primarily based on the precipitation of soluble indicator dyes which become insoluble and colored zones or bands of enzyme activity (Wendel and Weeden 1989). Recognisable banding patterns are produced on gels which allows an organism to be fingerprinted based on the genetic coding for the different enzymes (Micales *et al.* 1992). Enzymes coded by different genetic loci usually occur in separate bands of the gel which facilitates genetic interpretation (Bonde *et al.* 1993). With many isolates the amount of intraspecific variation determined by isozyme analysis is slight thus making accurate identification of a species or subspecies possible (Bonde *et al.* 1993) and unlike other molecular techniques, such as random amplification of polymorphic DNA (RAPDS) analysis which is capable of detecting minute changes in the genome of an organism, it detects only significant differences in enzyme structure (Bonde *et al.* 1993). These differences most often occur at the species level and so allow a group of isolates from the same species to be distinguished.

A preliminary screening run of the isozyme methods (Micales 1986) was undertaken to determine if it could be used as an additional technique to separate sterile fungi into taxonomic groups. Isozyme analysis requires a screening experiment to determine which buffer systems should be used for the best electrophoretic separation of each enzyme and to show which enzymes can be extracted in sufficient quantity. The determination of the correct buffer is essential as an inappropriate buffer can result in the absence of staining (caused by the wrong pH) and poor band resolution (Bonde *et al.* 1993). For this screen, standardised protocols for isozyme electrophoresis were used (Soltis *et al.* 1983).

Three isolates from five sterile groups and six isolates from a sixth group (Table 2.2) were grown on VBM (Appendix 1) in liquid culture by the same method given previously (2.2.1 (h)). After 14 days growth, each liquid culture was centrifuged (10 000 min^{-1},10 min), the supernatant broth was discarded and the remaining mycelium freeze dried (Flexi-dry MP). Freeze dried mycelium was ground in a chilled mortar and stored in Nunc cryo

tubes at -80°C until required. Each isolate was also grown on PDA for 7 -14 days before electrophoresis.

Table 2.2 Sterile fungal isolates used for Isozyme analysis.

Sterile Group	Strain Code	Original host
Sterile Dark Group 3	1 SV2WNB1P5S3-1366	Sweet vernal
	2 C2WSB1P9S3-1414	White clover
	3 SV2WS1P8S5-1385	Sweet vernal
Sterile Dark Group 5	1 SV1WN2P4S2-188	Sweet vernal
	2 C1WS7P6S3-988	White clover
	3 B2WNB1P7S4	Browntop
Sterile Dark Group 7	1 R2WS1P2S5-1338	Ryegrass
	2 B2WNB1P1S3-1209	Browntop
	3 R1R6.2P30S9-713	Ryegrass
Sterile Dark Group 8	1 C1WN3P5S1-2002	White clover
	2 R2WNA1P6S4-1370	Ryegrass
	3 B2WS3P6S5-1270	Browntop
Sterile Hyaline Group 1	1 SV2WNB1P12S5	Sweet vernal
	2 C1WS9P4S6	White clover
	3 R2WS1P7S4	Ryegrass
	4 SV1WN1P7S8	Sweet vernal
	5 SV2WS3P7S5	Sweet vernal
	6 SV2WNB1P11S2	Sweet vernal
Sterile Hyaline Group 3	1 SV2NA2P11S3	Sweet vernal
	2 B2WS2P5S3	Browntop
	3 B2WS1P1S4	Browntop

In preparation for horizontal electrophoresis, the freeze dried samples were resuspended in Nunc tubes in 0.3 ml of phosphate extraction buffer (Appendix 6). Mycelium from each isolate was also scraped off the surface of 14 day PDA cultures and suspended in 0.3 ml phosphate extraction buffer (pH 7.5). Mycelial suspensions were stored overnight at 4°C. The mycelial extracts were absorbed on filter paper wicks (2 x 13 mm) for 15 min at 4°C, which were then loaded onto 12.8% hydrolysed starch gels (Sigma) by inserting each wick in a vertical slice cut at one end of the gel. Wicks soaked in homogenised mouse liver extract were also loaded as controls, and wicks soaked in bromophenol blue dye (Sigma) which were used as a marker lane and boundary front marker for the samples run on the gels. For electrophoresis, a 50 mA current was run through loaded gels until the bromophenol dye marker had migrated 9 cm, depending on the gel and buffer system used any enzyme bands produced will migrate varying distances behind the dye marker. The extract wicks were removed after 10 minutes of electrophoresis and the gels were kept cold during this process by the use of frozen slicker pads.

Following electrophoresis each gel was sliced horizontally (6 slices per gel) with a nylon string. Gel slices of both the annodal and cathodal ends were stained for enzyme activity following the published protocols (Soltis *et al.* 1983, Wendel and Weeden 1989). The enzyme names with enzyme commission code (E.C) and buffer systems are given in Table 2.3.

Electrophoresis of all isolates were run on five separate starch gel buffer systems: Amine-citrate (AC), Ridgeway (RW), Poulik (PK), Phosphate (PH), Tris-citrate (TC) (Appendix 6), and 18 enzyme stains were evaluated for resolution and stainability (Table 2.3, Appendix 6).

Table 2.3 The 18 enzymes that were tested on sterile fungi isolates.

ENZYME SYSTEMS	E.C. No.
Aspartate aminotransferase (AAT)	2.6.1.1
Adenylate kinase (AK)	2.7.4.3
Creatin kinase (CK)	2.7.3.2
Esterase (EST-A)	3.1.1.1
Glutamate dehydrogenase (GDH)	1.4.1.3
Glyceraldehyde-3-phosphodehydrogenase (GPD)	1.2.1.12
Glucose-6-phosphate isomerase (GPI)	5.3.1.9
Glucose-6-phosphate dehydrogenase (G6P)	1.1.1.49
Glutamate-ammonia ligase (GUS)	6.3.1.2
Isocitrate dehydrogenase (ICD)	1.1.1.42
Leucine aminopeptidase (LAP)	3.4.11
Lactate dehydrogenase (LDH)	1.1.1.27
Malate dehydrogenase (MDH)	1.1.1.37
Mannose phosphate isomerase (MPI)	5.3.1.8
Peptidase (PEP)	3.4
6-Phosphogluconate dehydrogenase (6PG)	1.1.1.44
Phosphoglucoisomerase (PGI)	5.3.1.9
Phosphoglucomutase (PGM)	5.4.2.2

2.2.4 TAXONOMY OF TWO FUNGI INDUCED TO SPORULATE AFTER INITIAL GROUPING AS STERILE MYCELIUM.

During the experiments undertaken to study and characterise the sterile fungi isolated from roots, some fungi that were initially sterile in culture were induced to sporulate and their morphologies were investigated further using light microscopy as well as scanning electron microscopy (SEM).

2.2.4 (a) *Thozetella tocklaiensis*

Single spore isolates from the sporulating cultures of SDG 10 were examined on PCA, HA, and OA. Dimensions of sporodochia, conidia, conidial setae, microawns, and

conidiogenous cells were determined using light microscopy. Measurements were based on 100 observations from each isolate on each medium.

Cultures were also examined using standard scanning electron microscope (SEM) techniques (Anderson, 1951, Cole and Samson, 1979). A sporulating isolate was cut from an axenic seedling plate and fixed in 6% glutaraldehyde and 1% osmium tetroxide (OsO_4) in 0.1 M sodium cacodylate buffer (pH 7.2) at room temperature for 1 hr. Samples were washed in 0.1 sodium cacodylate buffer (five changes over 30 min) and placed in 1% osmium tetroxide for 2 hrs. The samples were again washed in sodium cacodylate buffer (5 times over 30 min), dehydrated in 30, 50, 70, 90 and 95% acetone (15 min each), and then dehydrated in 100% ethanol (three changes, 15 min). Samples were critical point dried using CO_2 in a Denton DCP-1 drying apparatus, and sputter coated with 10 nm gold/palladium. Coated samples were examined in a Philips 505T electron microscope.

2.2.4 (b) *Phialophora* sp.

Light microscopy was used to examine two isolates of SHG 1 found sporulating on PCA slope cultures which had been stored at 4°C for nine months and then kept at room temperature for a further six weeks. Dimensions of conidia and conidiogenous cells were determined and these measurements were based on 100 observations from each isolate on each slope culture.

2.3 RESULTS

2.3.1 MORPHOLOGICAL EXAMINATION OF STERILE ISOLATES

2.3.1 (a) Classification of sterile isolates using cultural characteristics.

Sterile isolates grown on ten media exhibited varying colonial morphologies and growth rates. Descriptions are outlined below, and the mean radial growth of isolates was calculated for each group on each medium (Table 2.4)

Excluding isolates of SDG 10 and SHG 1, the majority of isolates did not sporulate on the range of media tested. YEA, PCA, SEA, V8 and SUC have all been used previously to induce asporogenous fungi to sporulate in culture (Sloan *et al.* 1960, Leach, 1962, Nemec, 1970, Hall 1985, Lowe pers. comm.) but this result was not repeated here and may indicate these isolates are truly sterile. Hall (1985, 1987) was able to identify sterile isolates as *Phialophora graminicola* and *Gaeumannomyces graminis* by inoculating sterile isolates onto SEA, this result was not achieved in this study as most sterile isolates in this study produced hyaline submerged colonies on this medium.

The transfer of isolates which occurred during the cultural examination led to the sub culture from nutrient poor media (such as the PCA storage slopes, HA) to nutrient rich media (such as MEA, SUC), these transfers provided so called 'nutrient shocks' to these isolates and this has method has been found to trigger sporulation in some species (Mordue IMI pers. comm.). This technique did not induce sporulation of any isolates.

Table 2.4 Mean radial colony diameters (mm) of sterile isolates on eleven different media incubated at 20°C after 14 days (or 6 days*).

Media	PDA	MEA	SEA	HA	PCA	V8	CPZ	SAB	YEA	SUC	OA
SDG 1*	80+	80+	52 ± 2.5	80+	55.4 ± 3.2	80+	51.2 ± 4.1	80+	80+	80+	78.5 ± 1.2
SDG 2	32 ± 5.4	35.4 ± 2.8	17.4 ± 1.2	16 ± 2.2	26.3 ± 4.1	29.7 ± 5.2	27.1 ± 3.0	34.5 ± 0.9	38.0 ± 2.6	36.6 ± 4.4	31.0 ± 1.9
SDG 3	16.8 ± 0.4	15.0 ± 1.5	12.0	6.2 ± 0.7	8.0 ± 1.2	15.2 ± 2.0	5.0 ± 0.1	12.4 ± 2.3	15.2 ± 3.0	13.7 ± 1.5	17.2 ± 1.3
SDG 4	58.8 ± 2.5	61.4 ± 4.7	40 ± 1.9	52.4 ± 2.0	57.3 ± 3.3	66.2 ± 3.2	57.1 ± 0.5	60.8 ± 6.4	59.0 ± 6.8	62.7 ± 4.9	59.5 ± 4
SDG 5	49.4 ± 4.1	50.5 ± 3.7	33.2 ± 4.2	45.7 ± 0.7	50.9 ± 5.6	54.3 ± 1	40.3 ± 2.9	49.9 ± 3.8	52.0 ± 2.0	46.2 ± 1.8	46.3 ± 3.3
SDG 6	80+	80+	71.4 ± 1.5	80+	80+	80+	80+	80+	80+	80+	80+
SDG 7	22.0 ± 5.5	29.3 ± 3.4	14.4 ± 2.8	16.2 ± 0.9	20.9 ± 4.9	16.6 ± 3.3	17.0 ± 2.4	25.2 ± 0.5	27.2 ± 4.9	28 ± 1.7	30.4 ± 2.0
SDG 8	9.9 ± 0.7	8.4 ± 1.4	9.9 ± 1.0	7.2 ± 2.1	8.0 ± 2.1	7.4 ± 1.2	3.0 ± 1.2	7.5 ± 0.6	5.1 ± 2.0	6.2 ± 0.7	8.0 ± 1.8
SDG 9	35 ± 3.2	35.2 ± 4.5	21.1 ± 2.2	20.5 ± 1.0	30.9 ± 0.3	37.7 ± 2.0	30.2 ± 0.8	33.3 ± 1.2	35.5 ± 3.5	38 ± 4.2	26.4 ± 4.5
SDG 10	20.0 ± 1	15.2 ± 3.1	11.4 ± 1.3	10.1 ± 3.0	10.2 ± 2.6	19.1 ± 3	14 ± 1.1	18 ± 1.3	15.7 ± 4.5	18.8 ± 2.3	11.2 ± 2.9
SHG 1	17 ± 0.9	15.4 ± 3.9	10.3 ± 1	12.8 ± 0.8	13.9 ± 4.5	22.6 ± 3.3	7.1 ± 3.8	19.0 ± 3.0	16.5 ± 4.0	18.6 ± 0.6	12.6 ± 2.6
SHG 2*	80+	80+	80+	80+	80+	80+	80+	80+	80+	80+	80+
SHG 3	16.6 ± 1.4	13.8 ± 1.3	11.1 ± 0.2	14.0 ± 0.3	15.0 ± 2.8	18.8 ± 1.1	7.2 ± 1.7	17.0 ± 2.9	17.0 ± 0.5	16.0	16.0 ± 1.4

*** isolates with rapid growth were measured after 6 days incubation before mycelium reached the edge of the plates, the remaining isolates were measured at day 14, ± standard deviation from the mean colony growth (mm).**

Cultural and morphological descriptions of sterile fungi plated onto ten media:

(i) Sterile dark group 1 (SDG 1)

Distinctive features.
Radial colony growth: Fast, 80 mm (after 6 days)
Mean hyphal diameter: 3-5 µm
Colony colour: grey
Reverse: Black

Colony growth diameter 80+ mm, very dense, grey (3 B1- D1), aerial floccose mycelium (Figure 2.1); except on SEA, PCA and CPZ where aerial hyphae were sparse, byssoid and hyaline with radial colony growth of 50-60 mm after 6 days. Reverse colony colour was black on all media apart from SEA, PCA and CPZ where it was hyaline.

(ii) Sterile dark group 2 (SDG 2)

Distinctive features.
Radial colony growth: Medium, 25-40 mm
Mean hyphal diameter: 3-5 µm
Colony colour: grey
Reverse: purplish grey
Produced chlamydospore-like cells

Colonies on all media 25-40 mm, very dense grey (3 C1 - E1) aerial floccose mycelium, Reverse colony colour purplish grey (14 D2). Some isolates produced sectors of darker or lighter grey funiculose hyphae, this differential melanization caused star like patterns in some cultures (Figure 2.2). Hyphal diameter 2-5 µm, swollen dark hyphal cells 5- 12 µm, which resembled chlamydospores were produced on PCA, PDA V8, HA, these cells were often aggregated together to form black tufts on the agar surface (Figure 2.2). Colonies on SEA, HA 15 - 20 mm, sparse lightly pigmented hyphae, submerged with no aerial mycelium.

(iii) Sterile dark Group 3 (SDG 3)

Distinctive features.
Radial colony growth: Slow, 10-18 mm
Mean hyphal diameter: 2-3 µm
Colony colour: Black
Reverse: Black

Colony growth varied according with medium:

Colonies on V8 8-15 mm diam., mycelium black, low, very dense with a smooth entire margin; moist yeast-like. Central colony growth undulate and rugose with deep

radial furrows (Figure 2.3). Although most isolates had a slimy yeast-like appearance, often the colonies would produce a thin smooth margin with a central area of dark brown floccose mycelia. Colonies on SEA 12 mm diam.; mycelium effuse with film-like growth, hyphae strongly appressed and submerged within the media, very fine aerial flocculose mycelia produced around the inoculation plug. Colonies on CPZ, PCA, HA 5-8 mm diam.; mycelium complanate (flat, smooth, moist, Figure 2.3), resembled the common black soil yeast-like species *Aureobasidium pullulans* var *melanogenum.* Colonies on SUC 3-5 mm diam, aerial mycelium black, velutinous, sulcate with irregular margins (Figure 2.4)

Isolates grown on the remaining media produced black colonies with a distinctive zonation of mycelial texture, a thin smooth sulcate margin, a dark velutinous central region and aerial floccose mycelium at the colony center. Most colonies had both radial and concentric furrows (Figures 2.3, 2.4).

Hyphal morphology also varied according to growth media, with nutrient poor media hyphae on SEA, PCA, CPZ, HA, 2-3 µm, closely appressed, parallel growth without lateral branching. Hyphae on remaining sugar rich media, monilioid 5-10 µm, darkly pigmented.

(iv) Sterile dark group 4 (SDG 4)

Distinctive features.
Radial colony growth: Medium 55 -70 mm
Hyphal diameter: 1.5-3.5 µm
Colony colour: Grey/Pink
Reverse: Grey/red
Orange exudate, soluble pigment

Colonies 55-70 mm diam. (except CPZ), growth varied on the different media:

Colonies on CPZ 35-40 mm diam. aerial mycelium floccose, with a concentric zonation colour pattern, which ranged from a greyish pink (10 B3) in the central part of the colony to a progressively lighter whitish pink (10 A2) at the outer part of the colony. Margins white, irregular and undulate. Orange (3 A8) exudate produced in droplets, a reddish brown (8 E8) soluble pigment also diffused into the agar on this medium (Figure 2.5). Reverse colony colour was a greyish red (9 B6). Colonies on PCA, HA 50-60 mm diam., aerial white floccose mycelium at the central part of the colony, outer colony mycelium smooth, flat with a cereous or waxy appearance. Margins white, entire, regular. Reverse colony colour greyish white (3 B1). Exudate and pigment production were absent. Colonies on SEA 40 mm diam., hyaline, flat, with submerged hyphal growth. Orange exudate and pigment absent.

Colonies on remaining media 55-70 mm diam., aerial mycelium floccose, greyish white (3 B1 - 3C1), reverse colony pinkish white (8 A2). Droplets of orange exudate

produced, soluble pigment absent on all media apart from OA. Hyphal diam. 1.5 -3.5 µm; all hyphae became progressively more dematiaceous as the cultures aged.

(v) Sterile dark group 5 (SDG 5)

Distinctive features.
Radial colony growth: Medium 45-55 mm
Hyphal diameter: 2-5 µm
Colony colour: Dark brown
Reverse: black
Mycelial strands, submerged growth appressed hyphal branching.

Colonies 45-55 mm diam., mycelium submerged, complanate, dark brown (8 F4, Figure 2.6), limited aerial mycelium floccose particularly on the sugar rich media (PDA, MEA, SUC, SAB, YEA). Colony reverse black. Smooth regular entire margins. Hyphae melanized dark brown to black depending on age of the culture. Hyphal diam. 2-5 µm, hyphal branching closely appressed. Mycelial strands comprising 3-5 hyphae observed on all media. Colonies on SEA 33 mm diam., mycelium completely immersed, hyaline but with sectors of darkly melanized hyphae which formed irregular zones of pigmentation in most isolates.

(vi) Sterile dark group 6 (SDG 6)

Distinctive features.
Radial colony growth: Fast 80+ mm
Hyphal diameter: 2-5 µm
Colony colour: Dark grey
Reverse: black
Monilioid hyphae, cells

Colonies reached 80 mm within 10 days, aerial mycelium on sugar rich media dark grey (7 F1) to black, floccose (Figure 2.7). Clear droplets of exudate often produced by hyphae on some media (PDA, YEA, MEA, SUC, OA), which gave a metallic grey sheen to these colonies. Hyphae often formed darker mycelial strands on the agar surface forming rivulose patterns within the floccose mycelium. Colonies on nutrient poor PCA and HA 80 mm diam. mycelium submerged sparse lighter grey (7 C1) pigmentation (Figure 2.7). Colonies on SEA 70 mm diam. mycelium completely immersed within the agar, small rust brown crystalloid structures were deposited in a dendritic pattern within the media.

Most isolates had pleomorphic colony growth in culture which was due to variations in pigmentation, sectoring patterns and inconsistent production of mycelial strands, therefore these isolates were often hard to group on the basis of cultural morphology.

Mean hyphal diameter 3-7.5 µm, and hyphae possessed both intercalary and terminal swellings. These swollen hyphal cells were constricted at the septa but remained attached

which produced a distinctive type of monilioid branching. These swollen cells and lobes ranged from 10-20 µm to 10-40 µm. Hyphal branching was irregular as distance between branch origins and septa was variable, this coupled with irregular monilioid hyphae and lobes meant there was no regular growth pattern. Tufts of aggregated monilioid hyphal cells formed dark chlamydospore-like bodies on some cultures.

(vii) Sterile dark group 7 (SDG 7)

Distinctive features.
Radial colony growth: Slow/Medium 15-32 mm
Hyphal diameter: 2-5 µm
Colony colour: Dark brown
Reverse: dark brown
Dendritic mycelial strands, submerged growth, cylindrical chlamydospore-like cells .

Mycelium submerged, complanate, differentiated zones or sectors of dark brown (6 F8) and light brown (6 D5) pigmentation occurred on many colonies. Colony reverse dark brown (6 F8). Margins irregular and pale. Colony growth rates varied according to media; CPZ 5 mm, V8 15-18 mm, PCA 18-24 mm, and 20-30 mm diam. on remaining media. Colonies on SEA 12-16 mm, submerged, hyaline, hyphal diameter 0.5-1.5 µm; black crystalloid structures produced a maculate or spotted appearance. Concentric zones of crystal production in the media also created unique colony patterns on this medium.

Colonies composed of mycelial strands (also called fascicles) 10-16 µm diam., which formed dematiaceous rivulose or dendritic patterns (Figures 2.8, 2.9); hyphae 2-5 µm diam., irregular branching, and rugose. Cylindrical intercalary chlamydospores produced in long singular chains, finely rugose, slightly swollen at each end and 4-8 µm diam. (Figure 2.9), these structures were absent on HA, SEA, V8, YEA.

(viii) Sterile dark group 8 (SDG 8)

Distinctive features.
Radial colony growth: Slow 5-11 mm
Hyphal diameter: 2-3.5 µm
Colony colour: Olive green
Reverse: olive green-black
Soluble dark green pigment, velutinous sulcate colonies

Colonies 5-11 mm diam. (2-4 mm diam. on CPZ), colour greyish green (1C 5) to olive green (1F 8) in colony reverse dark green black, mycelium finely felted or velutinous texture, strongly cerebriform and often crateriform in central areas of colonies due to radial and concentric furrows (Figure 2.10). Margins sulcate and flat. A soluble extracellular yellow green (1B 8) to olive green pigment was produced which diffused into the agar (Figure 2.11) except on V8 where this pigment was absent. Hyphae 2-3.5 µm diam.,

monilioid hyphal cells 3 - 8 µm diam. (Figures 2.12, 2.13) Colonies on SEA 10 mm diam. mycelium submerged, hyaline complanate. Colonies also produced a whitish green centre with a submerged thin hyaline margin (Figure 2.10).

(ix) Sterile dark group 9 (SDG 9)

Distinctive features.
Radial colony growth: Medium 20-40 mm
Hyphal diameter: 2-4.5 µm
Colony colour: Black
Reverse: Black
White margins

Colonies 20-40 mm diam., similar to SDG 3, jet black, glabrous, moist, applanate (Figure 2.14). After 1 month colonies radially furrowed on all media apart from SEA, HA. Thin white, entire, 2 mm margins. Colonies on SEA 21 mm diam., submerged appressed. Hyphal growth was parallel, appressed and mostly immersed within the agar, mean hyphal diameters ranged between 2-4.5 µm on all media.

(x) Sterile dark group 10 (SDG 10 = *Thozetella tocklaiensis*)

Distinctive features.
Radial colony growth: Slow 12-22 mm
Hyphal diameter: 2-4.5 µm
Colony colour: Greyish brown
Reverse: Black
Concentric mycelial growth pattern

Colonies 12 - 22 mm diam. mycelium dense, aerial, floccose and grew in a concentric zonation pattern (Figure 2.15). Colonies on PDA, MEA, YEA, SUC, SAB, greyish brown (7 E5 - E1). Colony reverse reddish brown (8 C5). Colonies on CPZ 14 mm diam., aerial mycelium greyish white (7 B1), sparse and floccose. Colonies on HA, PCA, OA and SEA very slow (<16 mm diam.), mycelium, sparse, submerged, hyaline (Figure 2.16), hyphal diameter of 2-5 µm.

After exposure to UV light almost half these isolates produced sparse and discrete sporodochia on three media; HA, PCA and OA (Table 2.5, Figure 2.16), which eventually produced conidia after four-six weeks. A cold temperature shock and UV light also induced sporulation of all isolates on these media as well as two isolates grown on MEA. These isolates were the only sterile group which were induced to sporulate using these cultural incubation methods, although isolates remained sterile on all other media. Production of sporodochia allowed these isolates to be identified as the hyphomycete fungus, *Thozetella tocklaiensis* Agnihothrudu. The morphology of this fungus is was investigated in more detail in section 2.2.4 (a).

Table 2.5 Sporulation of *T. tocklaiensis*.

Media	PDA	MEA	SEA	HA	PCA	V8	CPZ	SAB	YEA	SUC	OA
UV +	-	-	-	++	++	-	-	-	-	-	++
UV -	-	-	-	-	-	-	-	-	-	-	-
2°C/UV+	-	+	-	+++	+++	-	-	-	-	-	+++

+++75-100% sporulation of isolates
++ 25-75% sporulation of isolates
+ <25% sporulation of isolates
- zero sporulation

(xi) Sterile hyaline group 1 (SHG 1 = *Phialophora* sp.)

Distinctive features.
Radial colony growth: Slow 14-22 mm
Hyphal diameter: 2-4.5 µm
Colony colour: White-Pale orange
Reverse: Pale orange/Peach
Cylindrical conidiophore-like cells, monilioid cells, exudate

Colonies on PCA, MEA, OA, HA 12-20 mm diam., mycelium moist, complanate, white to a distinctive pale orange (6 A3-7, Figure 2.17) Colony reverse also pale orange. Smooth hyaline margins. Colonies on PDA, SAB, SUC, YEA, 16-22 mm diam., central aerial mycelium dense and floccose, outer mycelium velutinous, sulcate with radial furrows, alutaceous orange brown (7C 7, Figure 18), colony reverse peach/ pale orange (7 A4); white submerged entire margins. Colonies on CPZ 1-2 mm diam., mycelium circular, veluntinous applanate white- pale orange (6 A3), with irregular white margins. Colonies on SEA 10 mm diam., mycelium submerged, hyaline, white margin absent (Figure 2.19). Colonies on V8 20-24 mm diam. central aerial mycelium floccose, outer mycelium velutinous, finely floccose or byssoid, pale yellow (4 A3) to white with a flat, white, entire margin. Colony reverse blood red (9 B8).

Colonies on PDA, OA, PCA, HA, MEA, YEA produced white atomate or powdery granular flanges which radiated from the central colony plug in a coralloid-like form. This white area appeared as though the colonies were sporulating (2.17). Minute fimbriate clear droplets produced in this region gave it a sparkly appearance (Figure 2.19). Exudate was produced on all media except V8 and copiously so on PDA, SUC and SAB. The outer area of the colonies were glabrous, flat and pale orange. Older 40 day old cultures became strongly convoluted with radial and concentric furrowing where copious exudate production accumulated.

Hyphae were hyaline with a diameter of 1-2.5 µm. Monilioid hyphae 4-10 µm diam. were produced on all media. The colony areas that appeared white and powdery were caused by the copious production of lateral outgrowth of multiseptate lobed branches from the main hyphae (Figure 2.18). These cylindrical outgrowths resembled conidiophores

particularly those of the *Oidiodendron* genus, but for the duration of this study, no spores were ever produced. These conidiophore like structures initially grew as simple swollen lobes but as growth developed branching became more complex, as side branches were produced in a dendritic pattern (Figure 2.18). These structures were up to 50 µm wide.

A selection of sterile isolates were maintained at room temperature as living cultures on PCA slopes in a test tube. At the conclusion of this study two of these isolates were belatedly discovered to be sporulating after 7 months growth in a storage slope. The production of collarettes and conidia from the previously described sterile cylindrical cells allowed these isolates and group to be identified as a species of *Phialophora.* The morphology of these isolates was studied in more detail and is described in section 2.2.4 (b)

(xii) Sterile hyaline group 2 (SHG 2)

Distinctive features.
Radial colony growth: Fast 80+ mm
Hyphal diameter: 4-6 µm after 6 days
Colony colour: White
Reverse: White
Clamp connections, clear exudate

Colony growth was rapid (> 80 mm after 6 days on all media) and aerial mycelium was dense floccose and white. Mean hyphal diameter 4-6 µm. Reverse colour was also white and droplets of clear exudate was produced on SUC and SAB. Mycelium was sparser on SEA but retained the characteristics described above. The distinguishing feature of this group was the production of clamp connections which identified these four isolates as basidiomycetes.

(xiii) Sterile hyaline group 3 (SHG 3)

Distinctive features.
Radial colony growth: Slow 12-20 mm
Hyphal diameter: 2-4 µm
Colony colour: white
Reverse: white
clear exudate, monilioid intercalary cells

Colonies on HA, OA, MEA and PCA 12-18 mm diam. mycelium white-hyaline complanate ceraceous, indistinct flat margins (Figure 2.20); colony reverse white; Colonies on CPZ 5-8 mm diam. mycelium same as that on PCA; colonies on SEA 12 mm diam., mycelium immersed, hyaline, black crystalloid bodies produced a black mottled lentiginous appearance (Figure 2.21). Colonies on V8, YEA, SUC, SAB and PDA 16-20 mm diam. aerial mycelium dense, white, floccose (Figure 2.20); colony reverse honey white (3 A2);

margins hyaline and submerged. Clear exudate was produced on PDA, SAB and SUC (Figure 2.20). Older colonies became sulcate after 30 days growth.

Hyphae were 2-4 µm in diameter and hyaline. Short lateral side branches were often produced as were hyphal swellings which were up to 5-10 µm wide (Figure 2.22). Many hyphae also produced intercalary swollen cells creating a monilioid bead-like appearance (Figure 2.22) these intercalary cells averaged 6 µm in diameter. These beaded cells also resemble the oidia produced by several *Oidiodendron* species (Domsch *et al.* 1980). Some hyphae aggregated together into tufts or balls and this was a common cultural characteristic exhibited by this group.

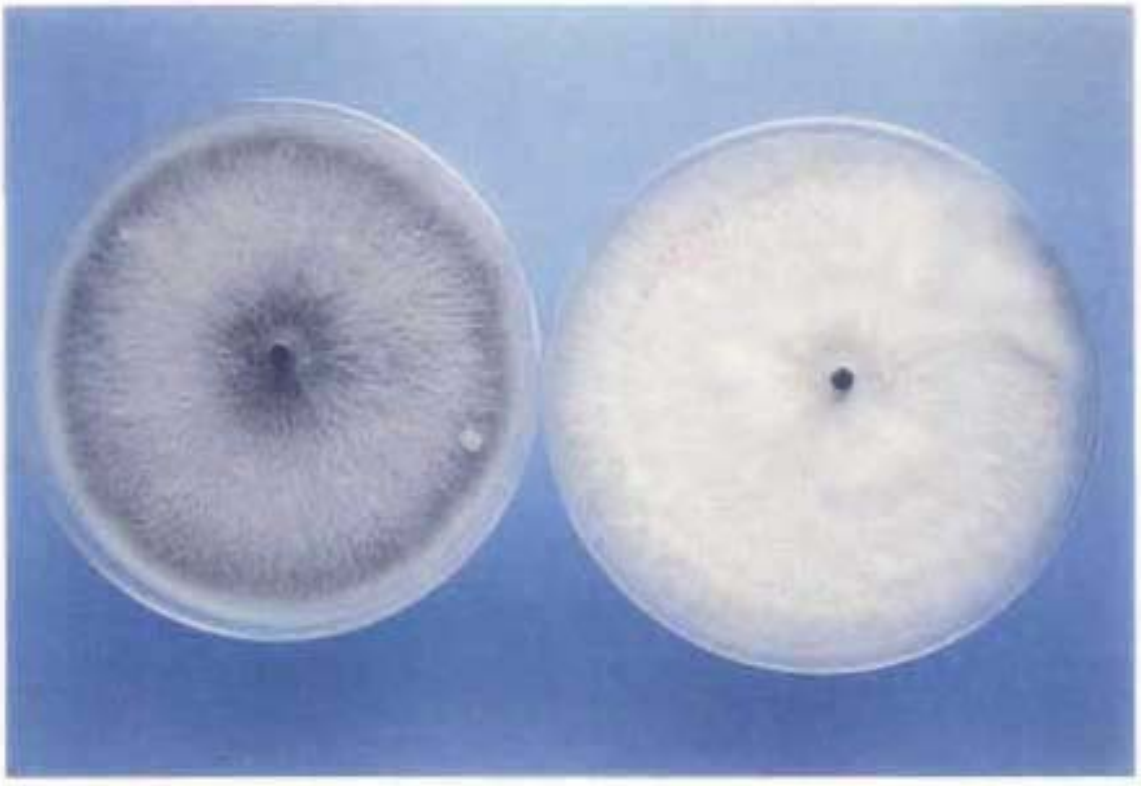


Figure 2.1 Sterile dark group 1, colonies on PDA (left), and PCA (right) after 6 days growth.

Figure 2.2 Sterile dark group 2, colony sectoring on PDA, (A) visible aggregated tufts of chlamydospore cells.

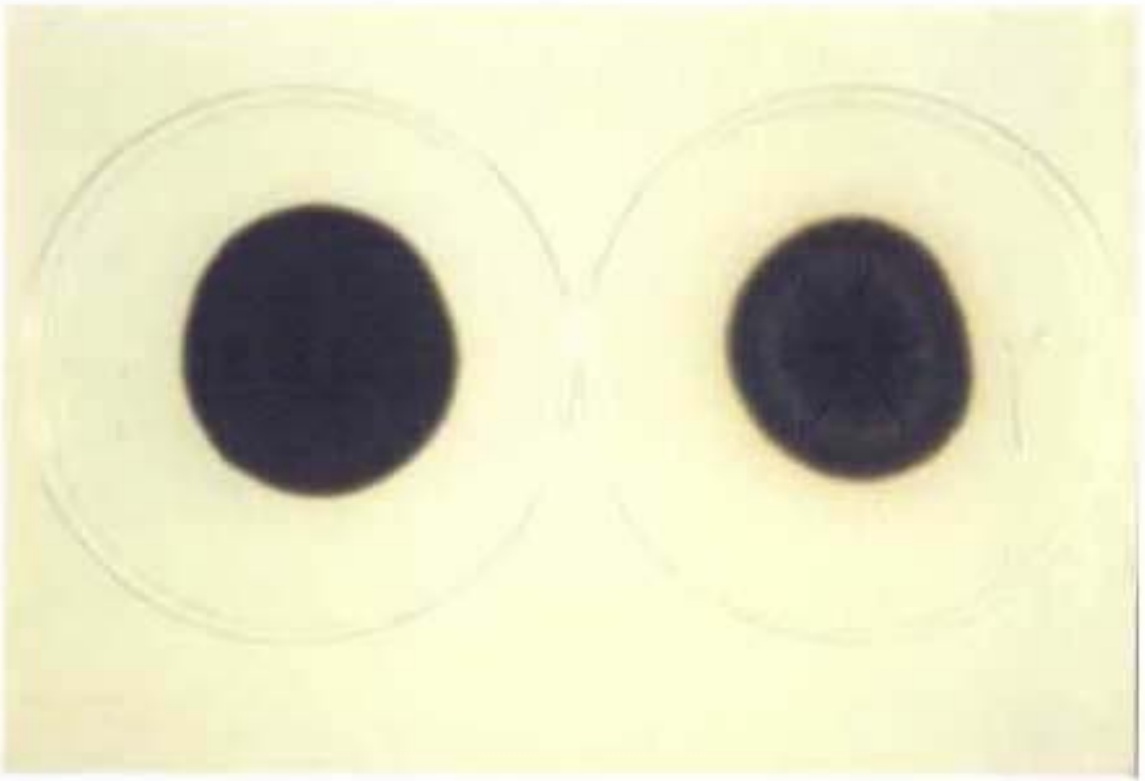


Figure 2.3 Sterile dark group 3 colonies on PCA (left) and PDA (right) after 21 days growth.

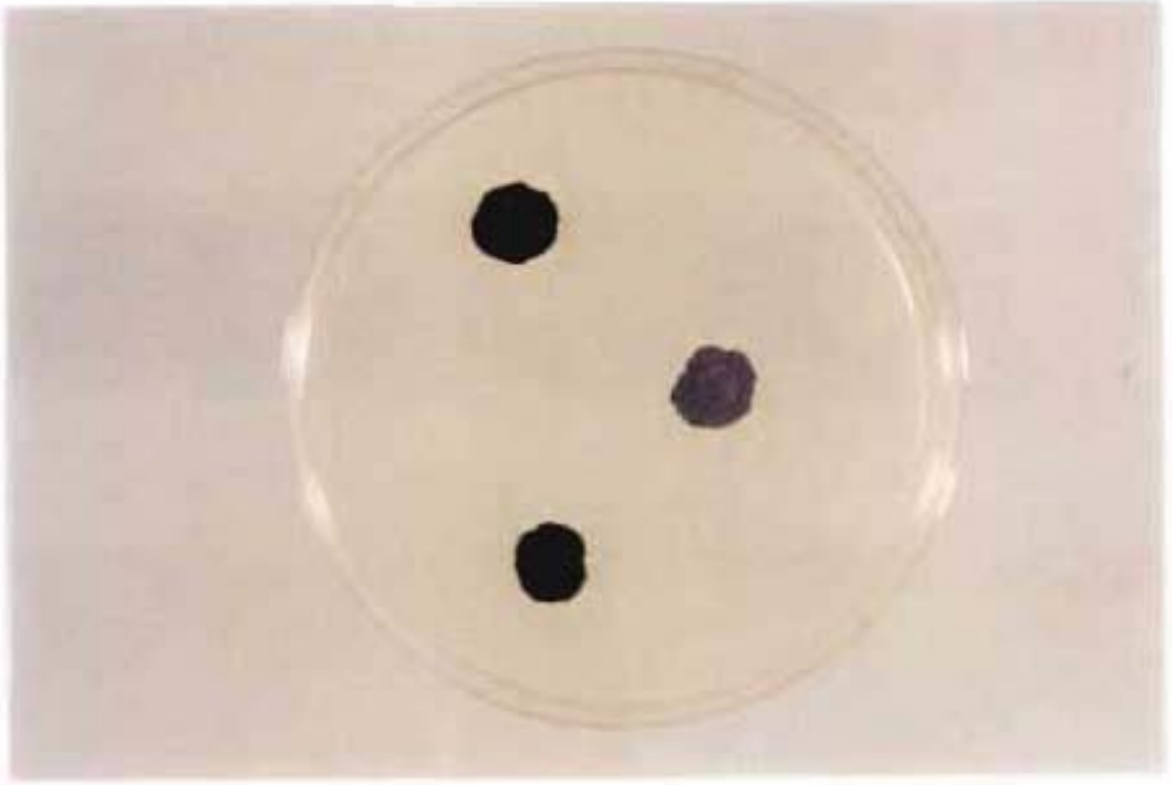


Figure 2.4 Sterile dark group 3 colonies on SUC after 14 days growth.

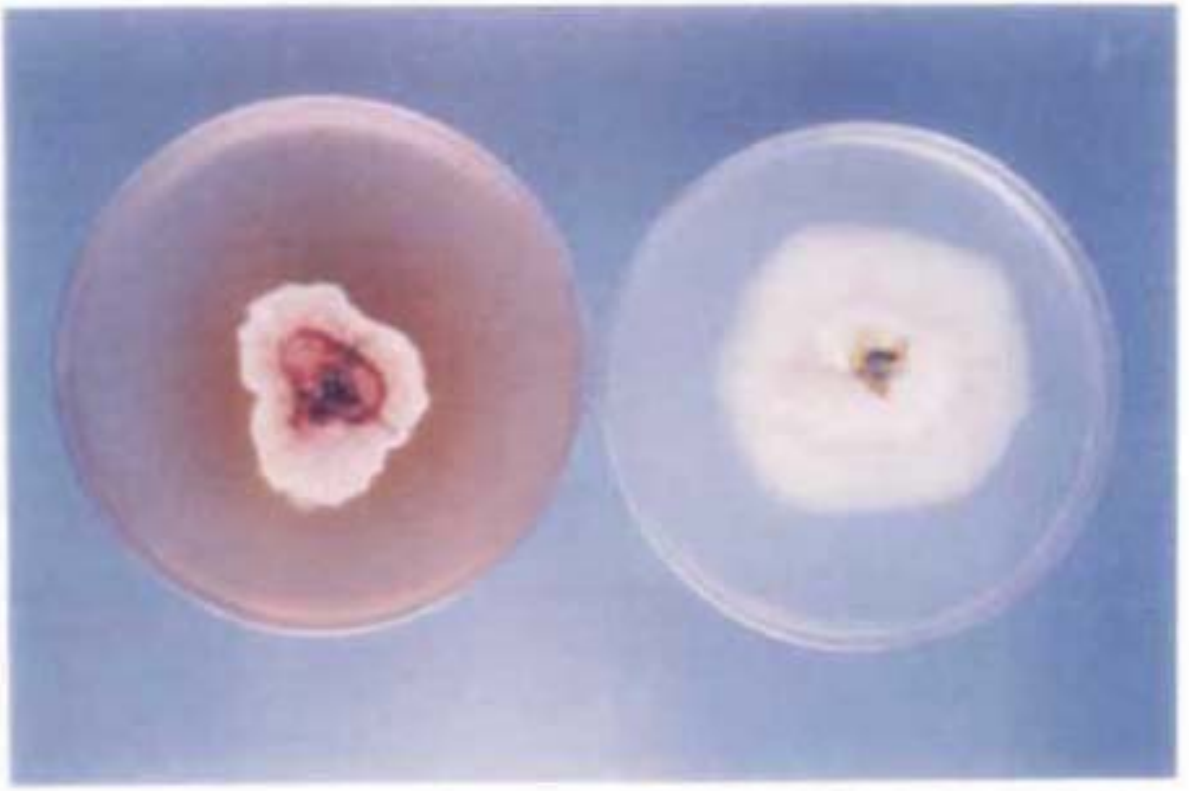


Figure 2.5 Sterile dark group 4 colonies on CPZ (left) and SUC (right) after 14 days growth.

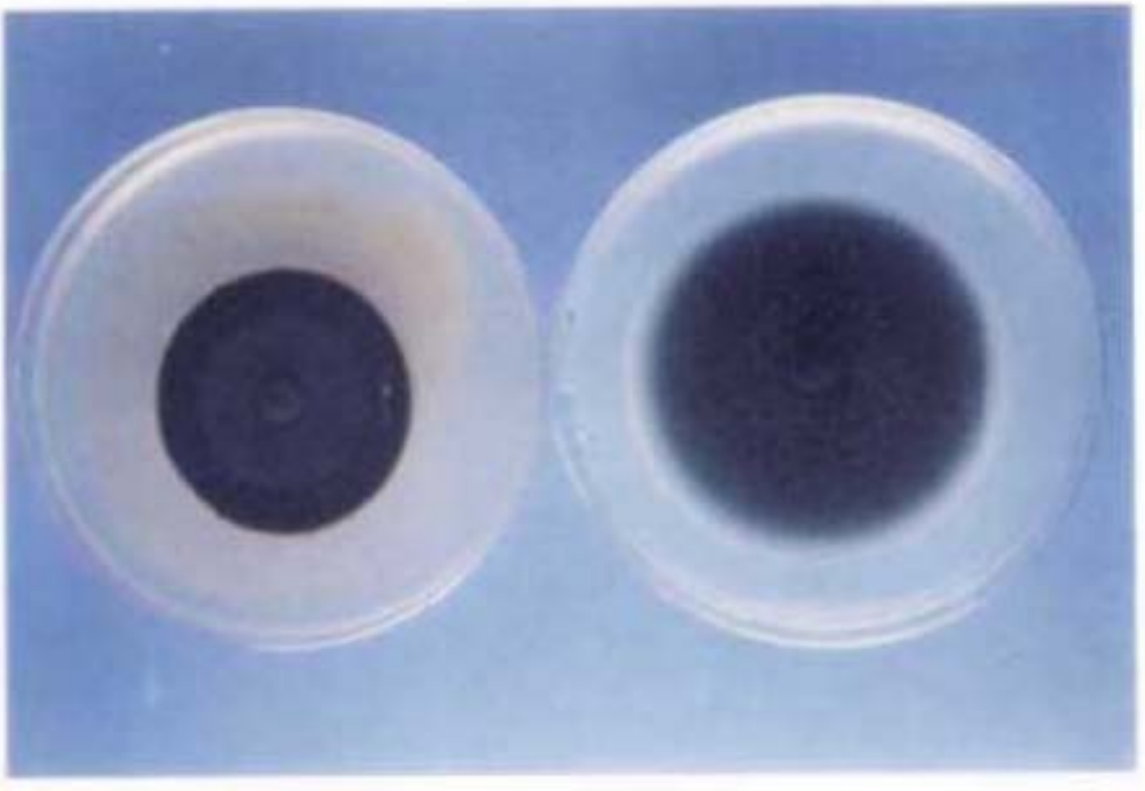


Figure 2.6 Sterile dark group 5 colonies on V8 (left) and PCA (right) after 14 days growth.

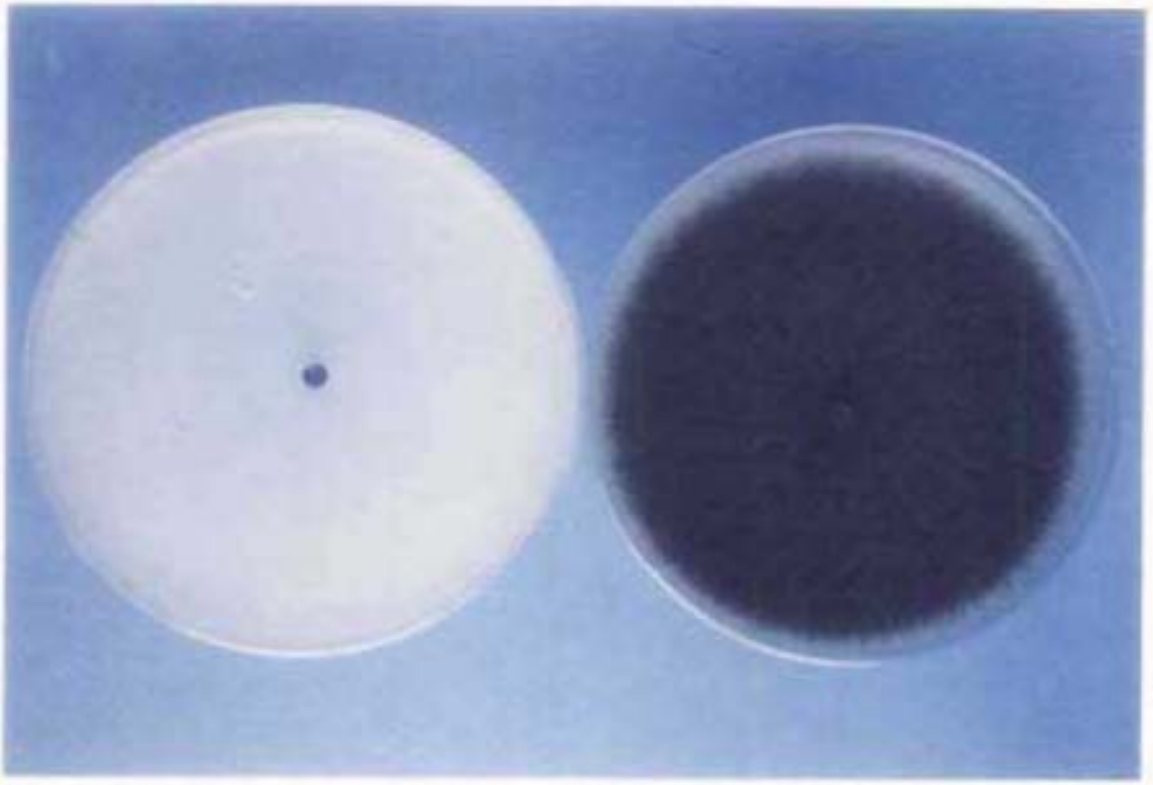


Figure 2.7 Sterile dark group 6 colonies on PCA (left) and PDA (right), after 14 days growth both colonies have reached the edge of the plate.

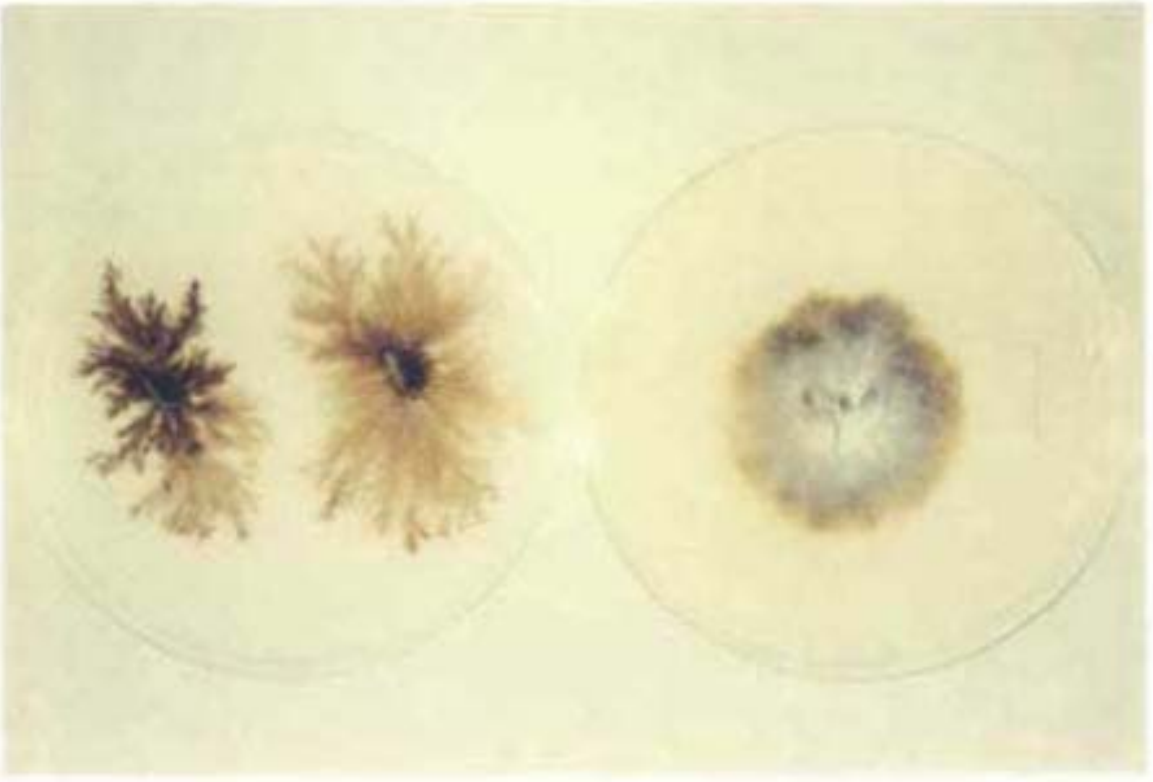


Figure 2.8 Sterile dark group 7 colonies on PCA (left) and PDA (right) after 14 days growth.

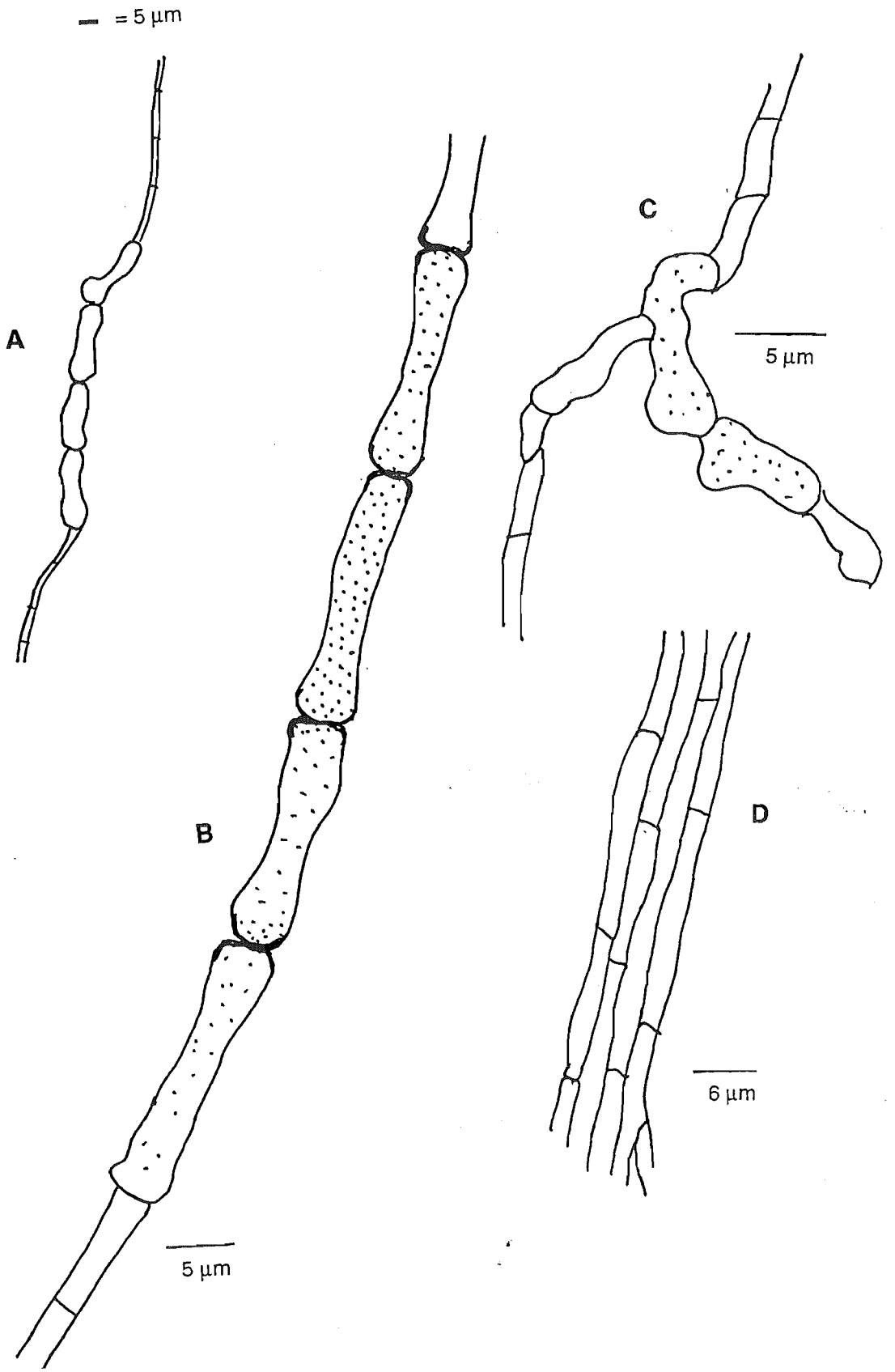


Figure 2.9 Sterile dark group 7, hyphal morphology (A) swollen intercalary cells, (B, C) pitted or rugose swollen hyphae (D) a mycelial strand or fascicle composed of 4 closely aggregated and individual hyphae.

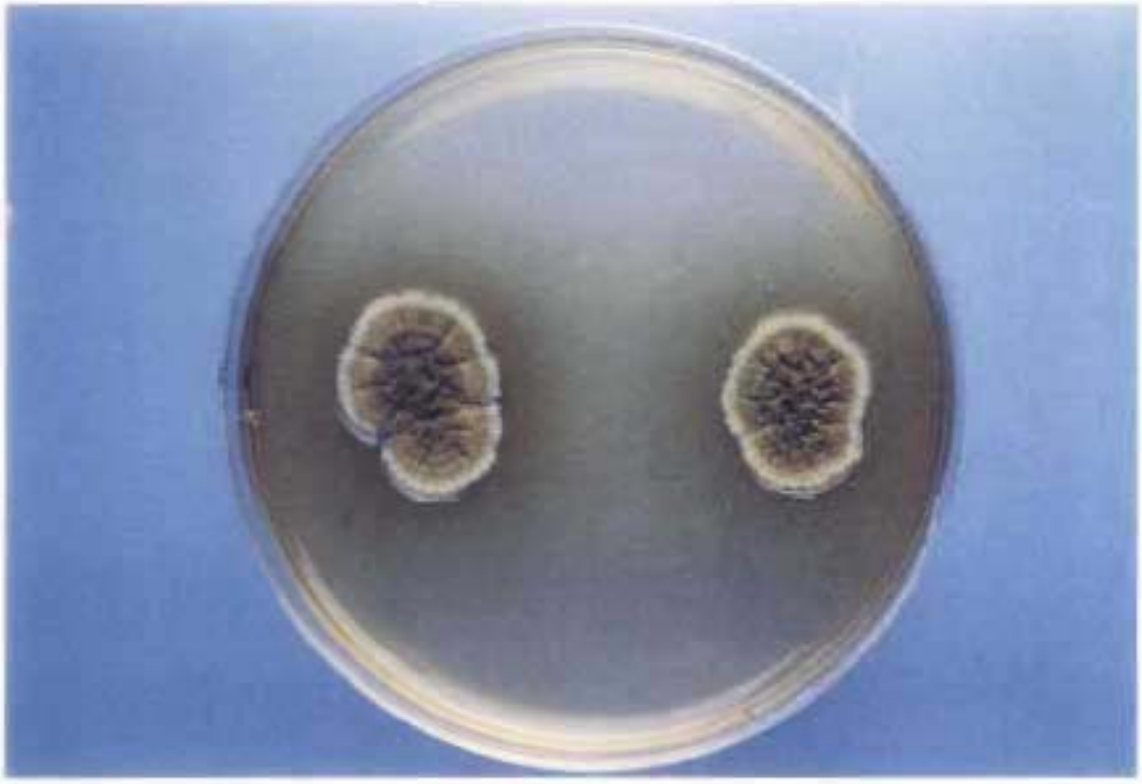


Figure 2.10 Sterile dark group 8 colonies on PDA after 30 days growth.

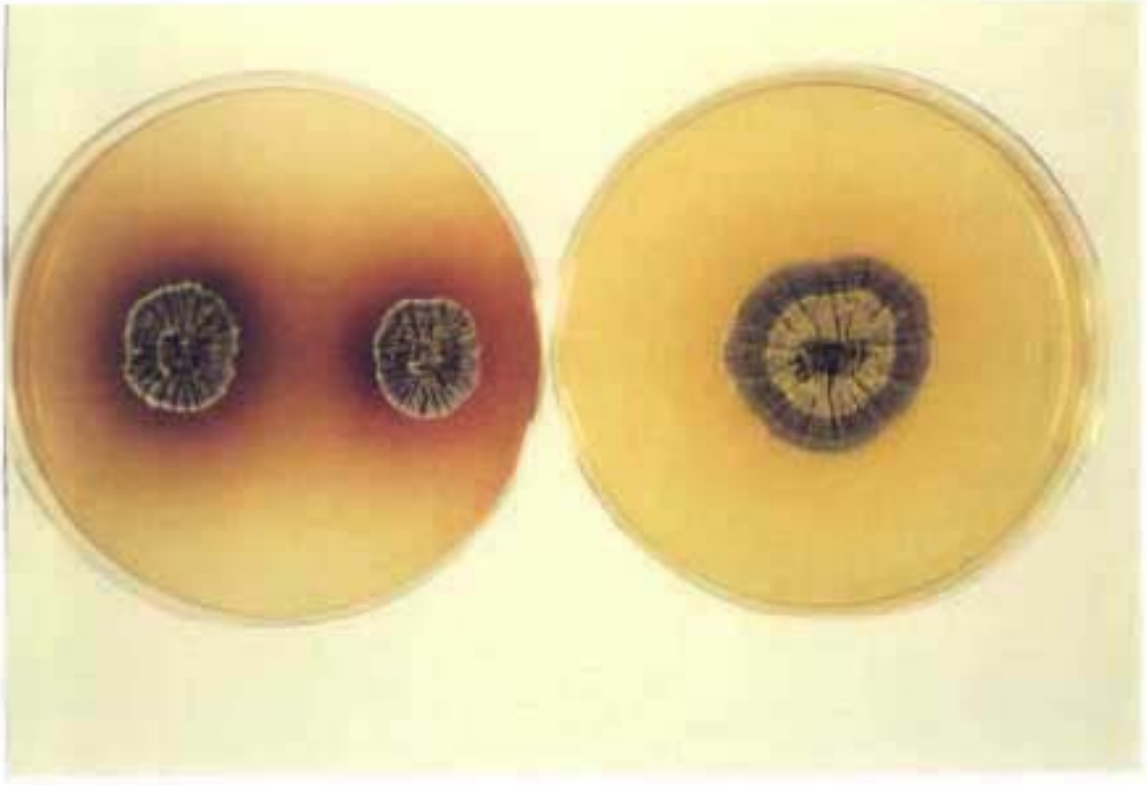


Figure 2.11 Sterile dark group 8 colonies on CPZ (left) and MEA (right) after 30 days growth.

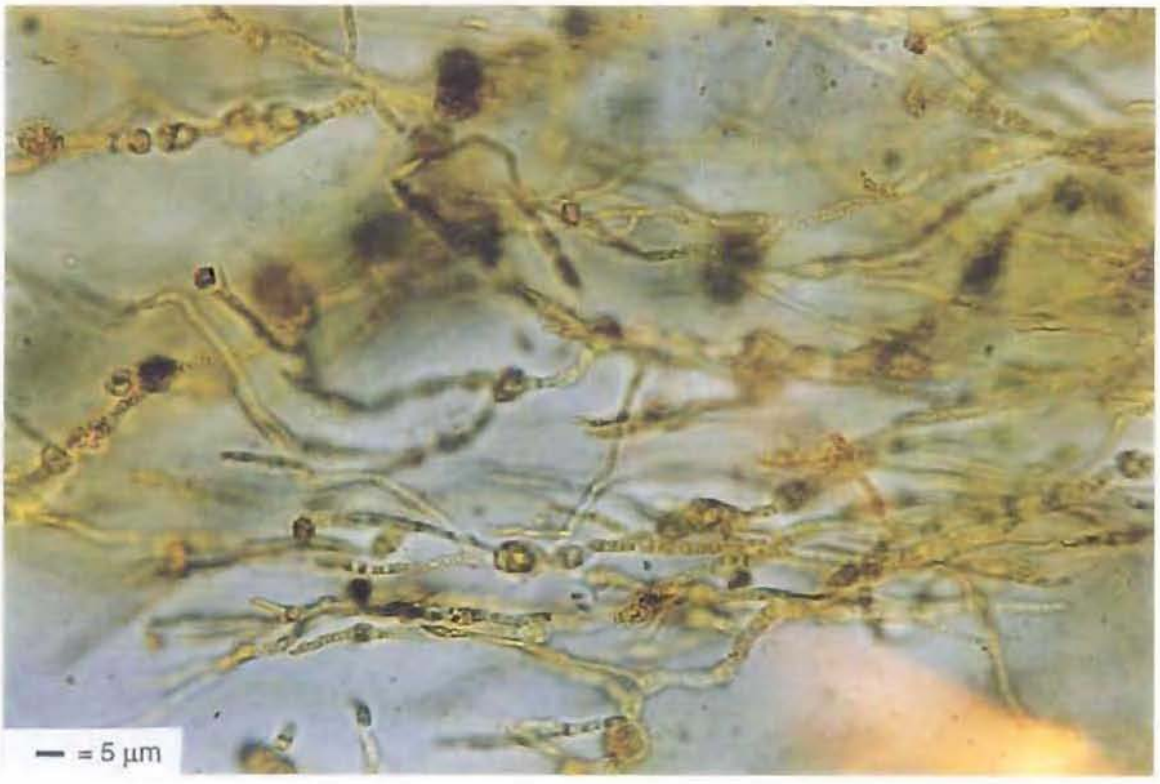


Figure 2.12 Hyphal morphology of sterile dark group 8 (400x magnification)

Figure 2.13 Hyphal morphology of sterile dark group 8 (A) monilioid cells (1000x magnification).

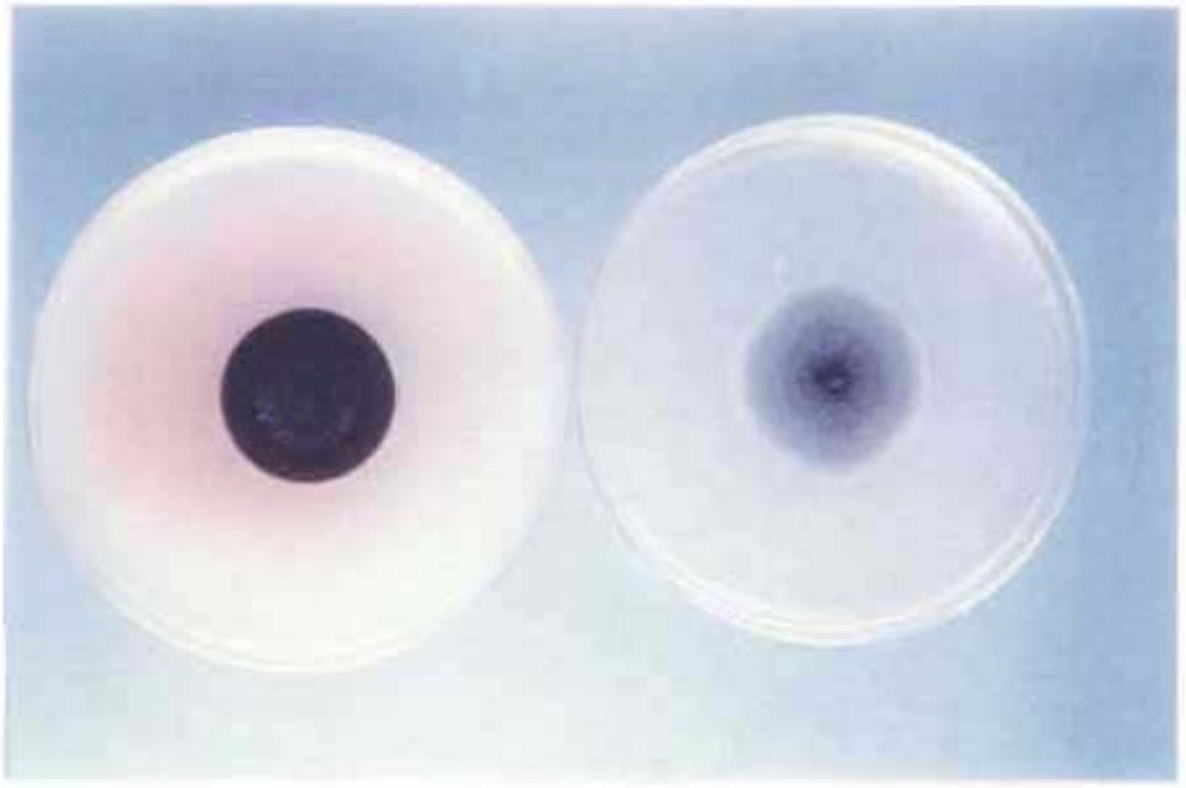


Figure 2.14 Sterile dark group 9 colonies on V8 (left) and PCA (right) after 14 days growth.

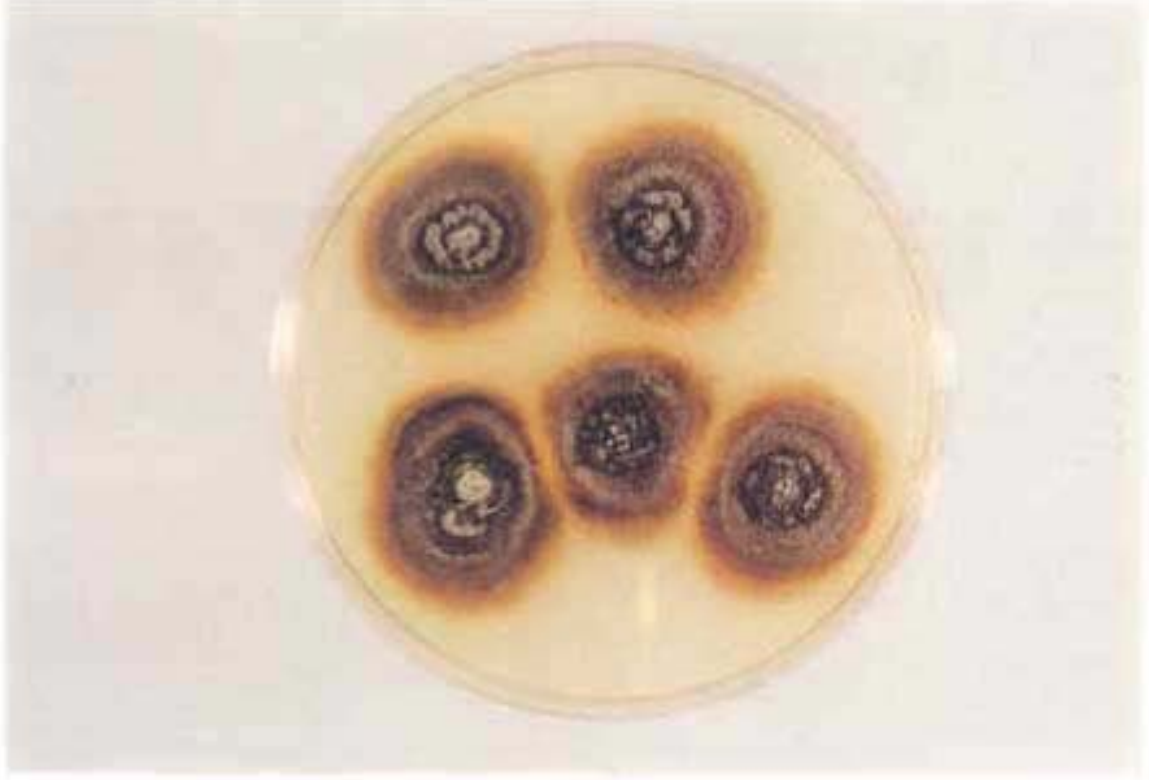


Figure 2.15 Sterile dark mycelium of *Thozetella tocklaiensis* on PDA after 21 days growth.

Figure 2.16 Hyaline mycelium of *Thozetella tocklaiensis* on OA after 21 days growth.

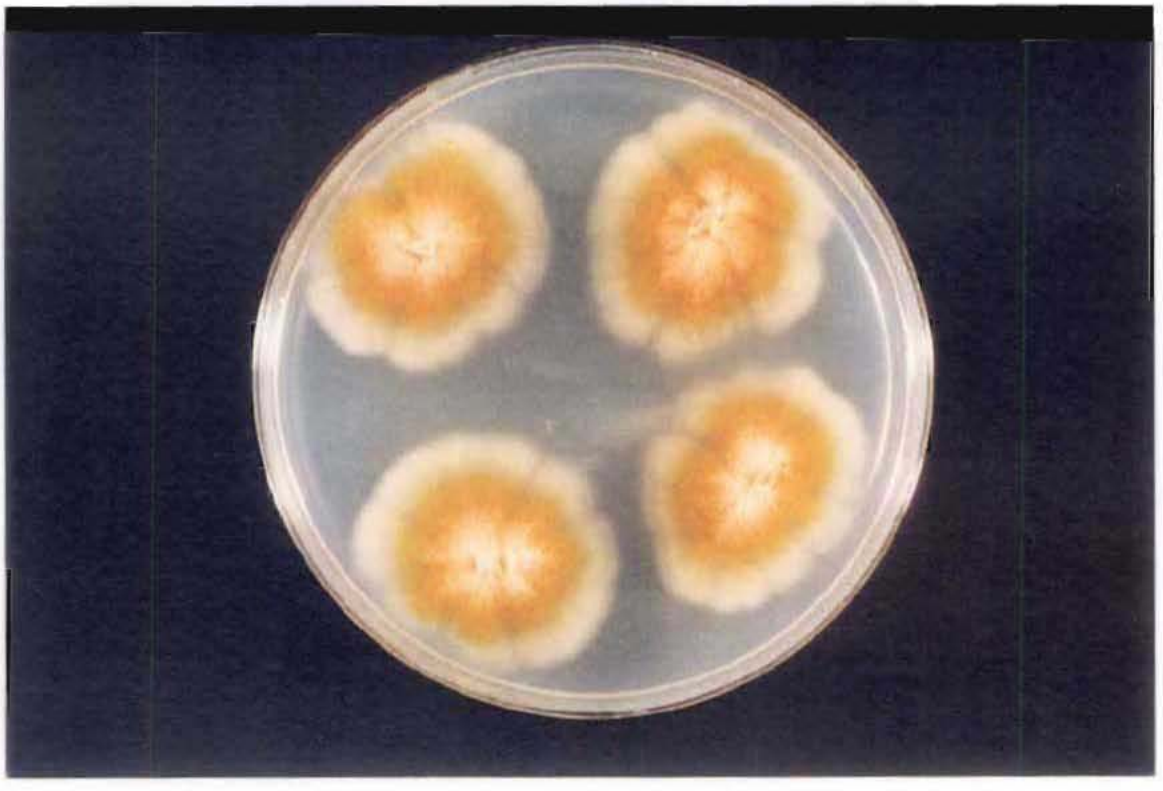


Figure 2.17 Sterile hyaline group 1 on PCA after 21 days growth.

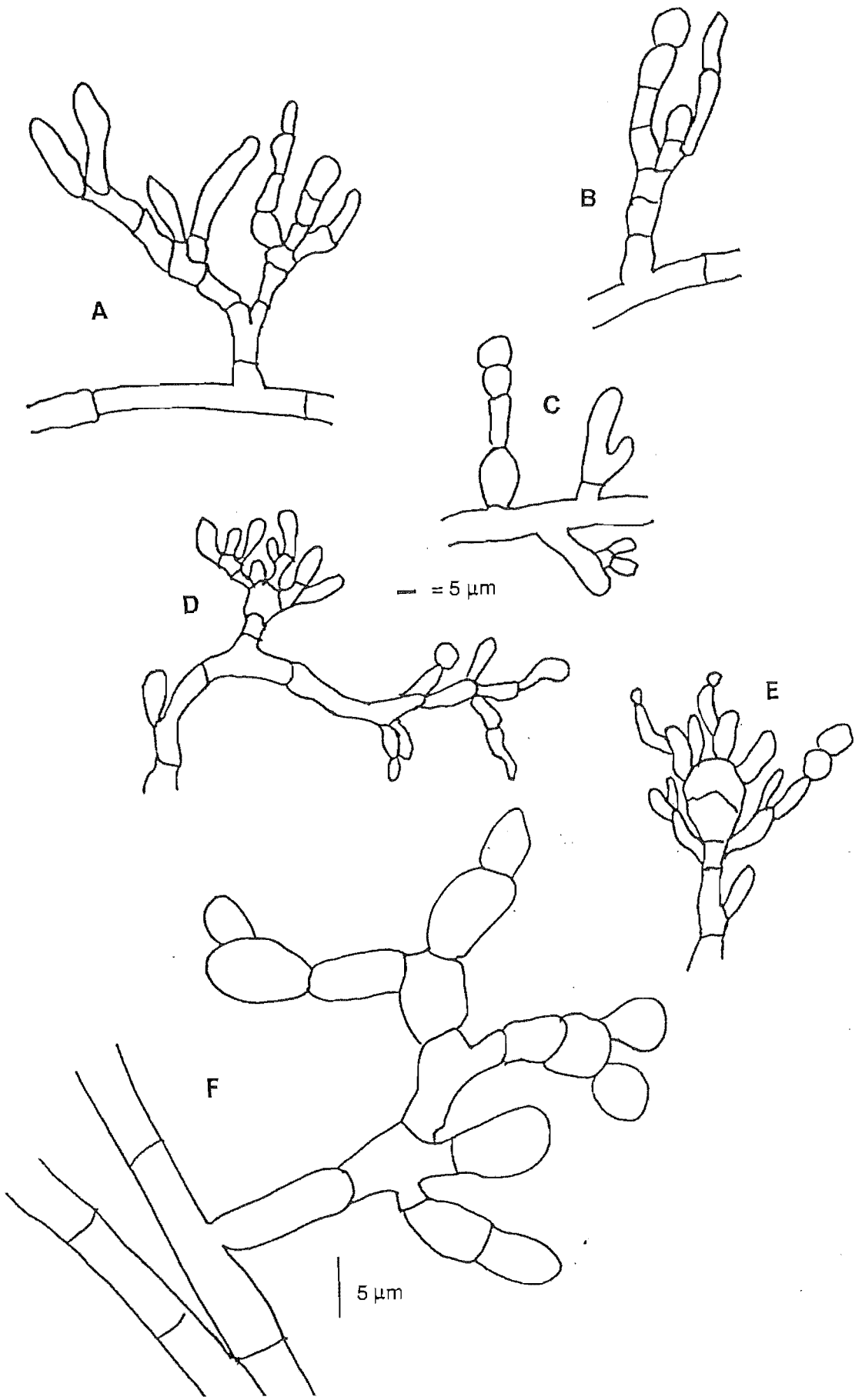


Figure 2.18 Sterile hyaline group 1, hyphal morphology (A-E) sterile conidiophore-like structures which initially grew as simple swollen lobes (C) but as growth developed became complex or dendritic (D, E), monilioid hyphae (F).

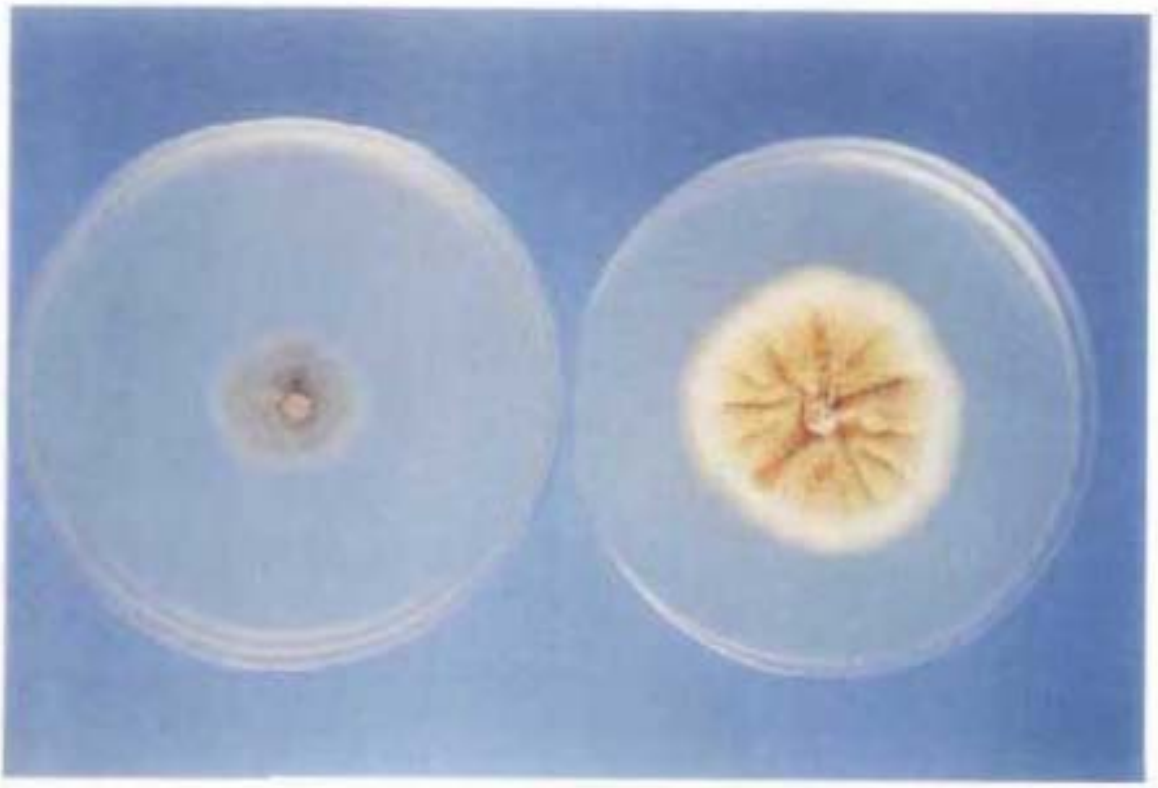


Figure 2.19 Sterile hyaline group 1 on SEA (left), and PDA (right) after 21 days growth.

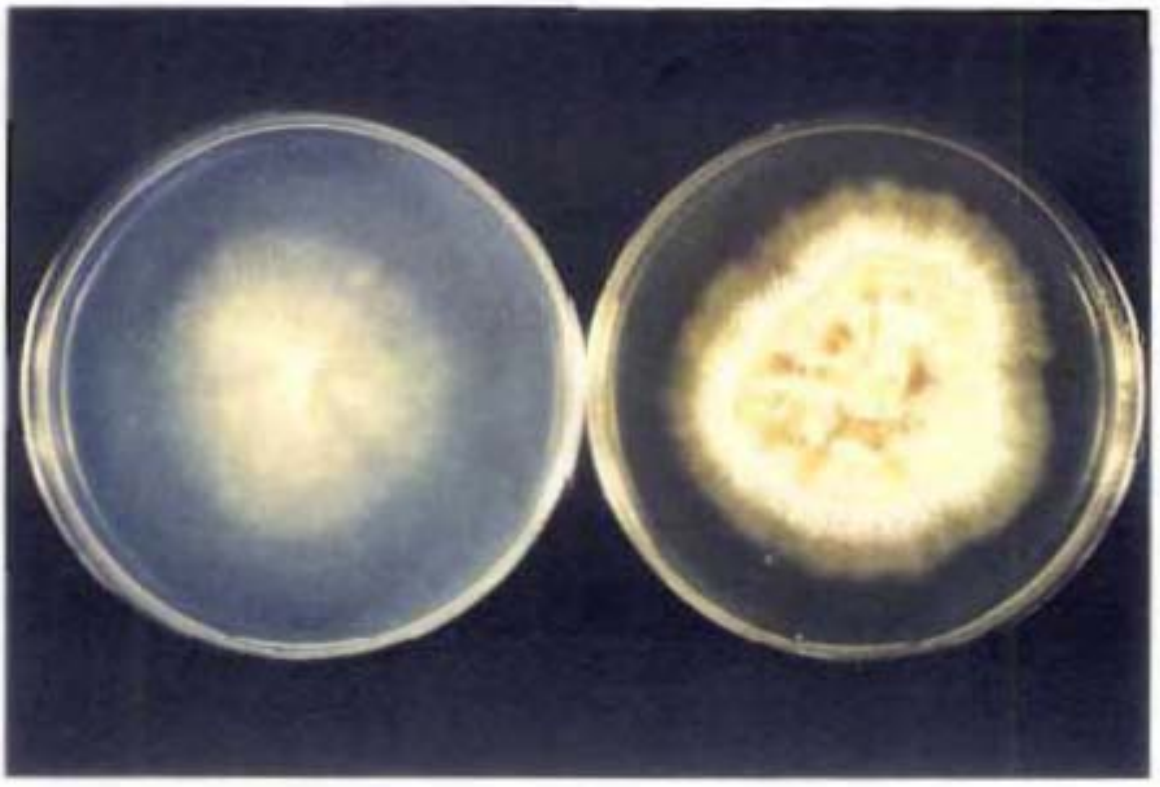


Figure 2.20 Sterile hyaline group 3 colonies on PCA (left) and PDA (right) after 21 days growth.

Figure 2.21 Sterile hyaline group 3 on SEA 12 after 30 days, mycelium immersed, hyaline and producing black crystalloid bodies.

Due to the volume of isolates and replication required in working with these sterile isolates, SDG 2,4,9 and SHG 2 were excluded from further investigation once the staining of nuclei and septa had been completed.

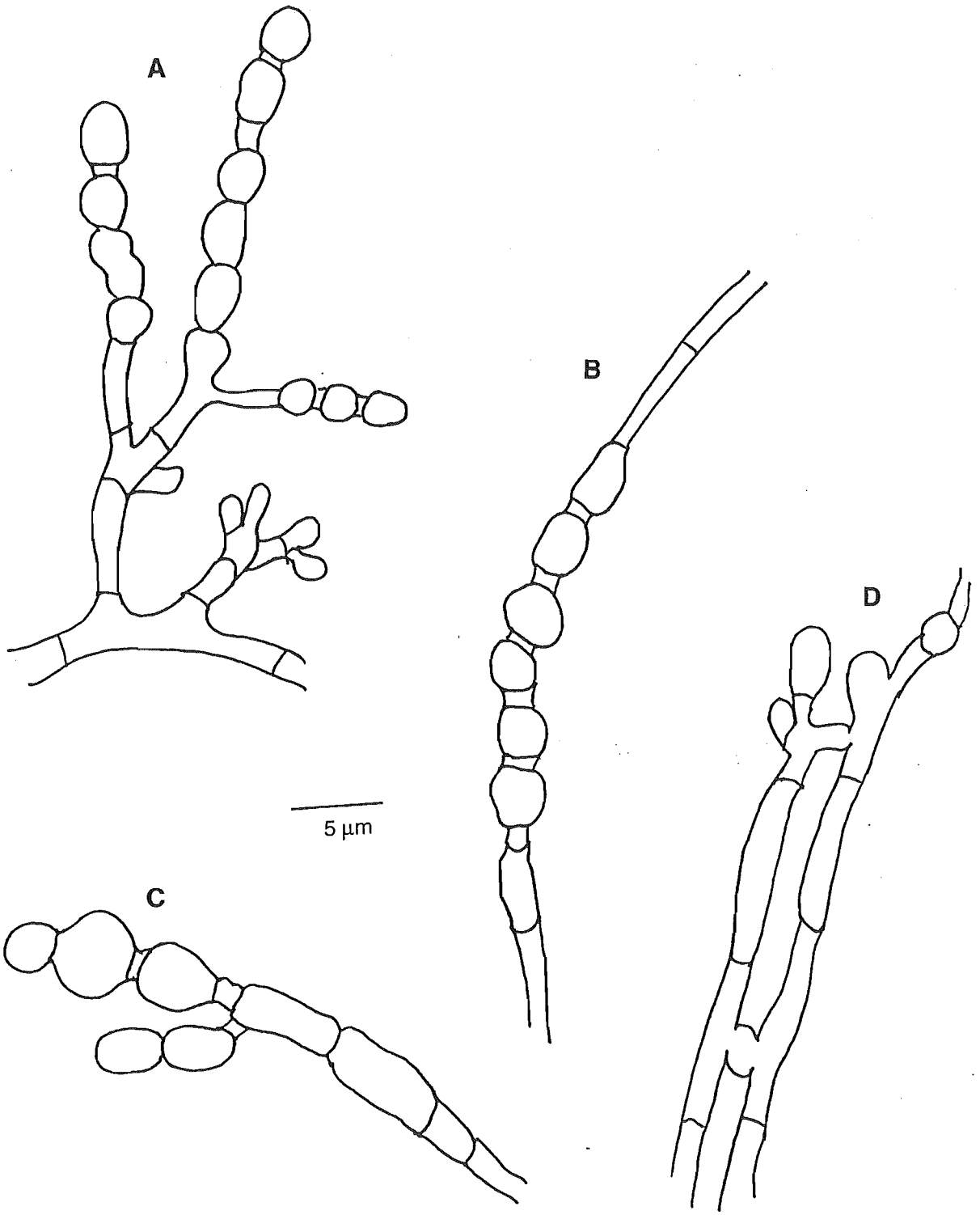


Figure 2.22 Sterile hyaline group 3, hyphal morphology (A-D) sterile terminal and intercalary swollen cells, conidiophore-like structures which initially grew as simple swollen lobes (A), monilioid hyphae (D).

2.3.1 (b) Nucleus and septum staining.

The staining of sterile isolates using two separate dyes gave a good contrast and clear view of both the nuclei and septae (Figures 2.23-2.31). The staining of nuclei with pH 7.8 Hoechst dye enabled the number per cell to be counted (Table 2.6), and most isolates contained 1 nucleus/cell but five sterile groups were multinucleate. Septa stained very well at pH 10.5 Hoechst dye (Figures 2.23-2.31), however no characteristic septation, such as dolipore septation, was observed. Isolates from the same sterile group had identical numbers of nuclei/cell which is additional evidence of a similar taxonomic grouping.

Table 2.6 Number of nuclei observed in sterile isolates

Fungal group	Number of nuclei
Sterile Dark Group 1	multinucleate (4-6/cell)
Sterile Dark Group 2	multinucleate (1-3/cell)
Sterile Dark Group 3	1/cell
Sterile Dark Group 4	multinucleate
Sterile Dark Group 5	1/cell (or some binucleate)
Sterile Dark Group 6	multinucleate (1-5/cell)
Sterile Dark Group 7	1/cell
Sterile Dark Group 8	1/cell
Sterile Dark Group 9	1/cell
Sterile Hyaline Group 1	1/cell
Sterile Hyaline Group 2	multinucleate (2-4/cell)
Sterile Hyaline Group 3	1/cell

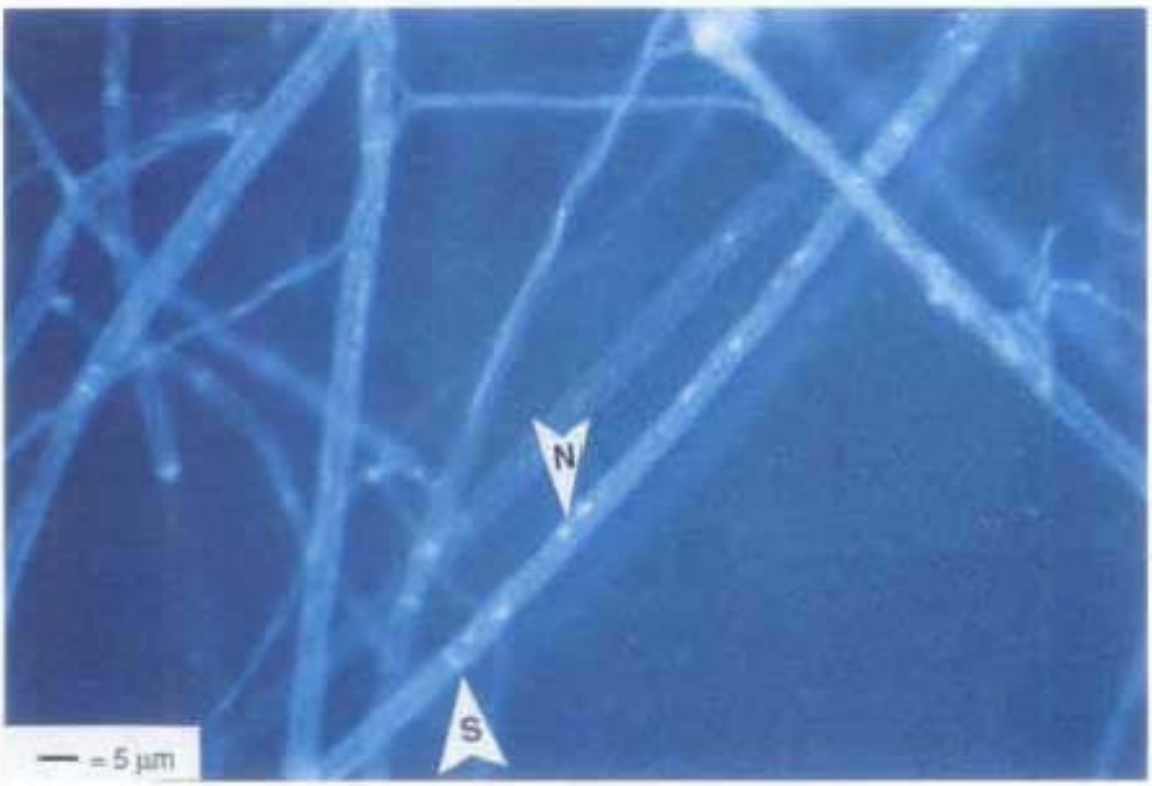


Figure 2.23 Stained nuclei (N) and septa (S) of multinucleate cells of SDG 1.

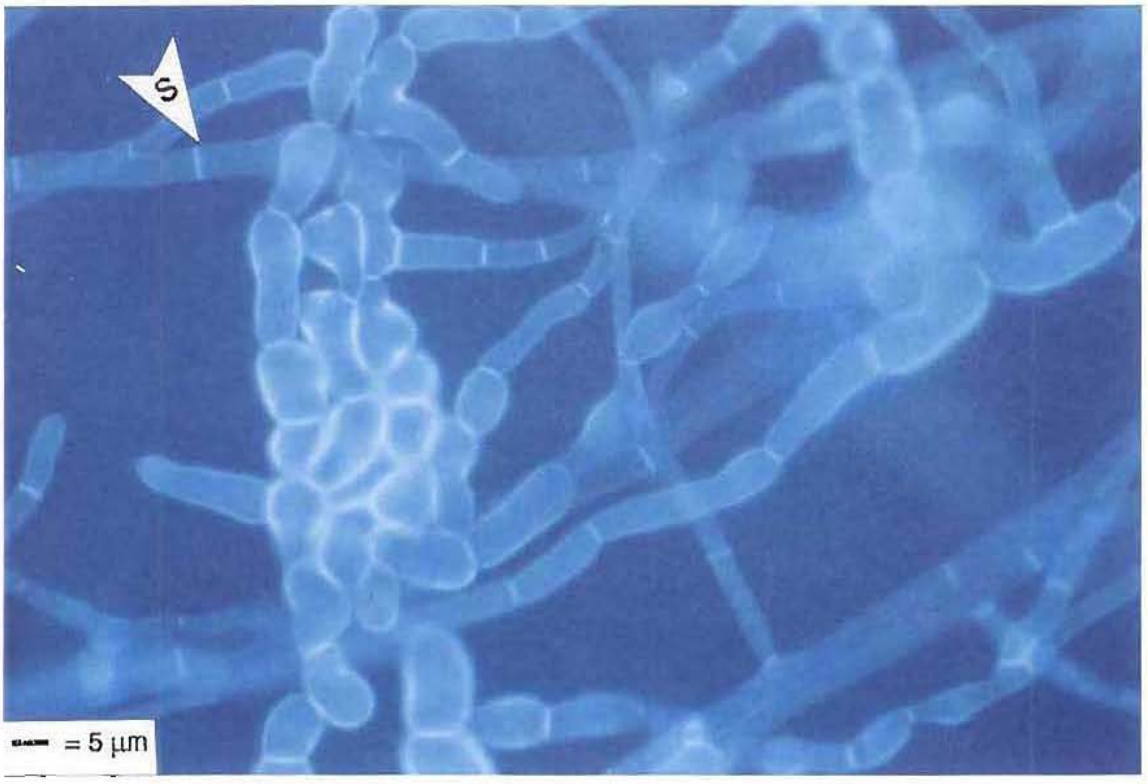


Figure 2.24 Stained septa (S) of SDG 5.

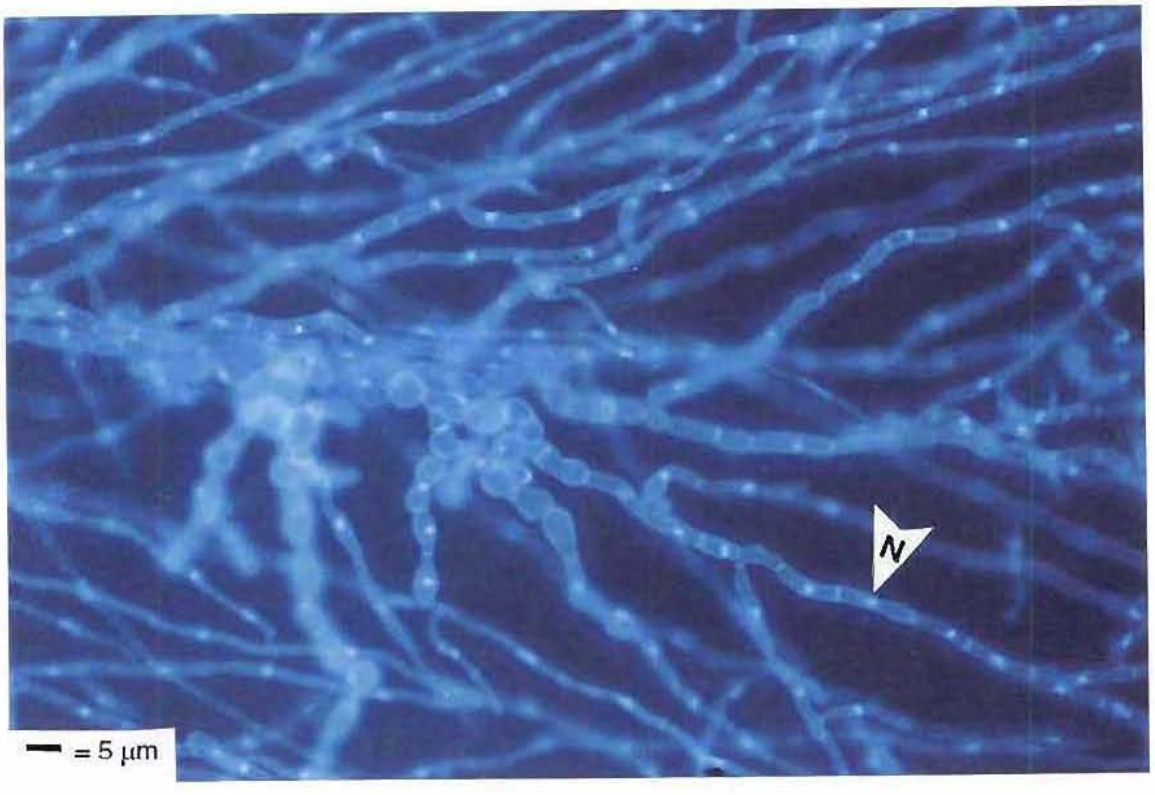


Figure 2.25 Stained nuclei (N) of uninucleate cells of SHG 1.

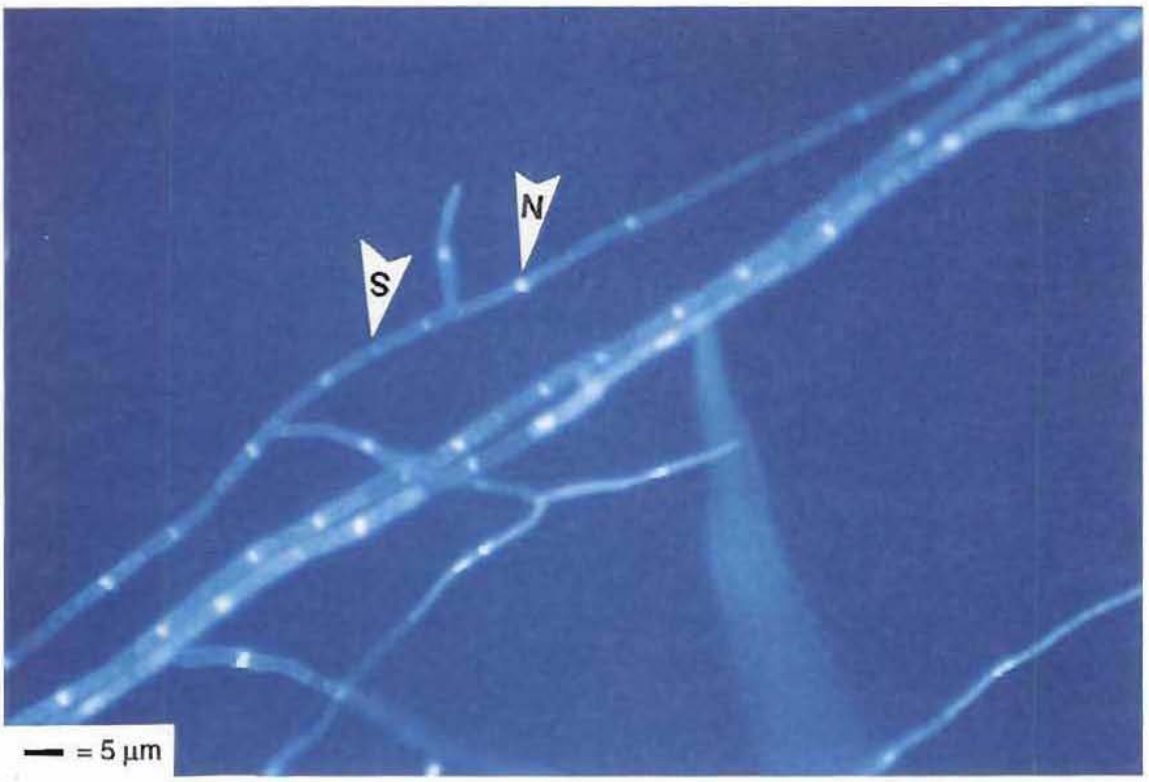


Figure 2.26 Stained septa (S) of SHG 1.

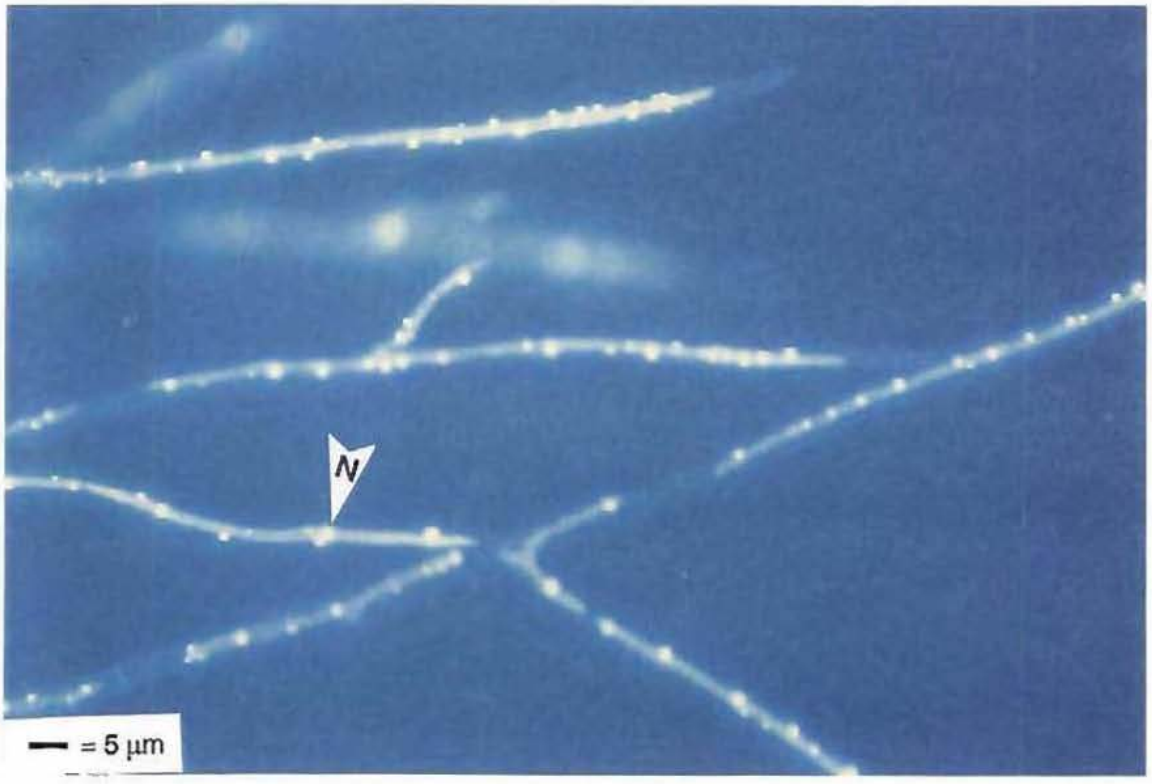


Figure 2.27 Stained nuclei (N) of multinucleate cells of SDG 4.

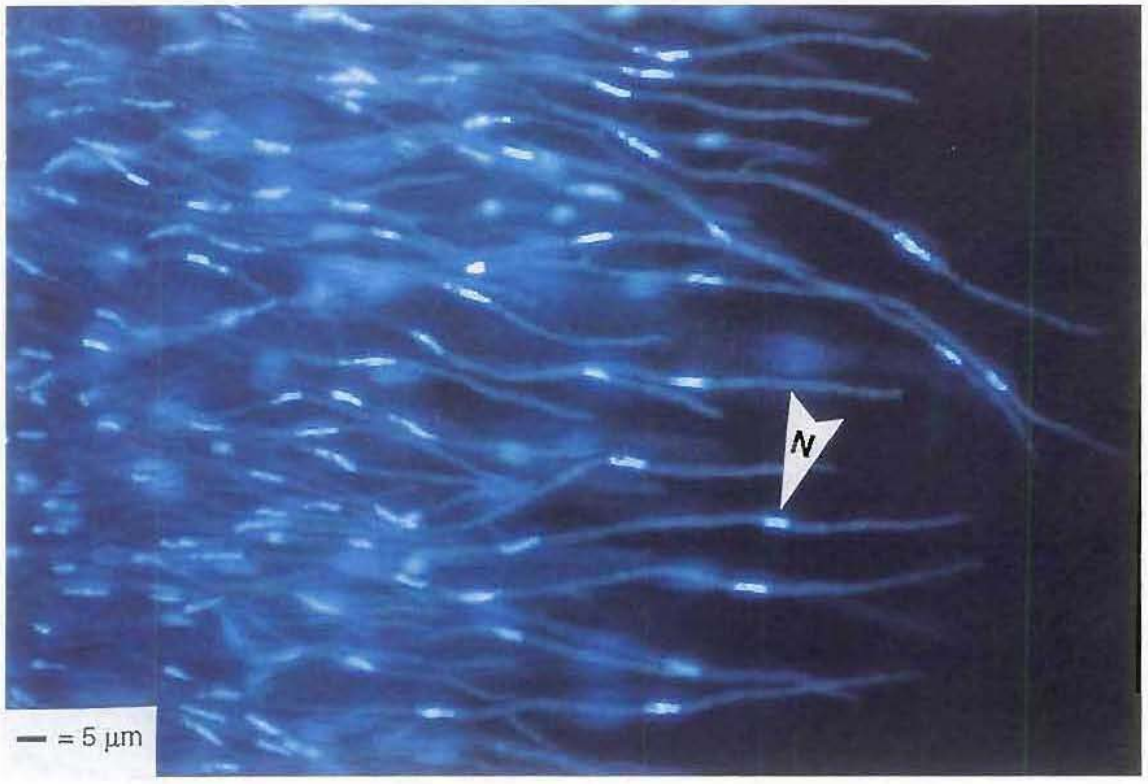


Figure 2.28 Stained nuclei (N) of uniuncleate cells of SDG 8.

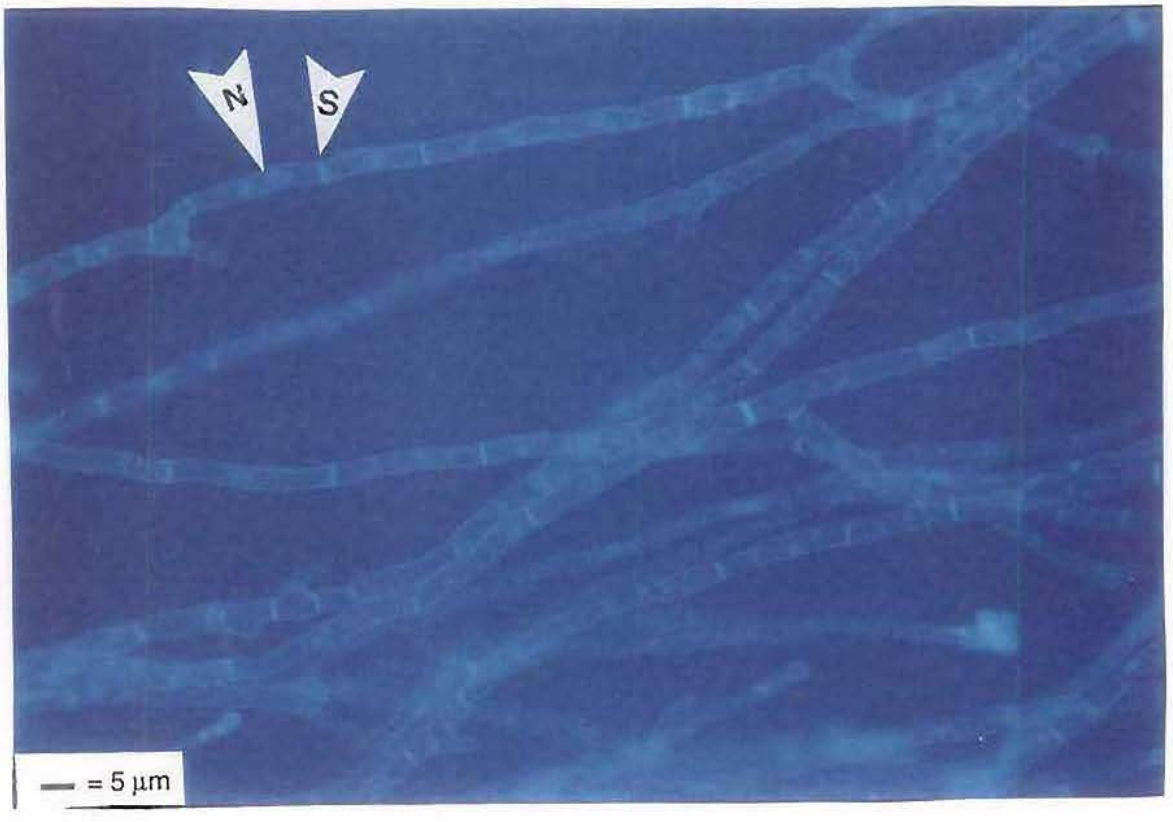


Figure 2.29 Stained nuclei (N) and septa (S) of multinucleate cells of SDG 6.

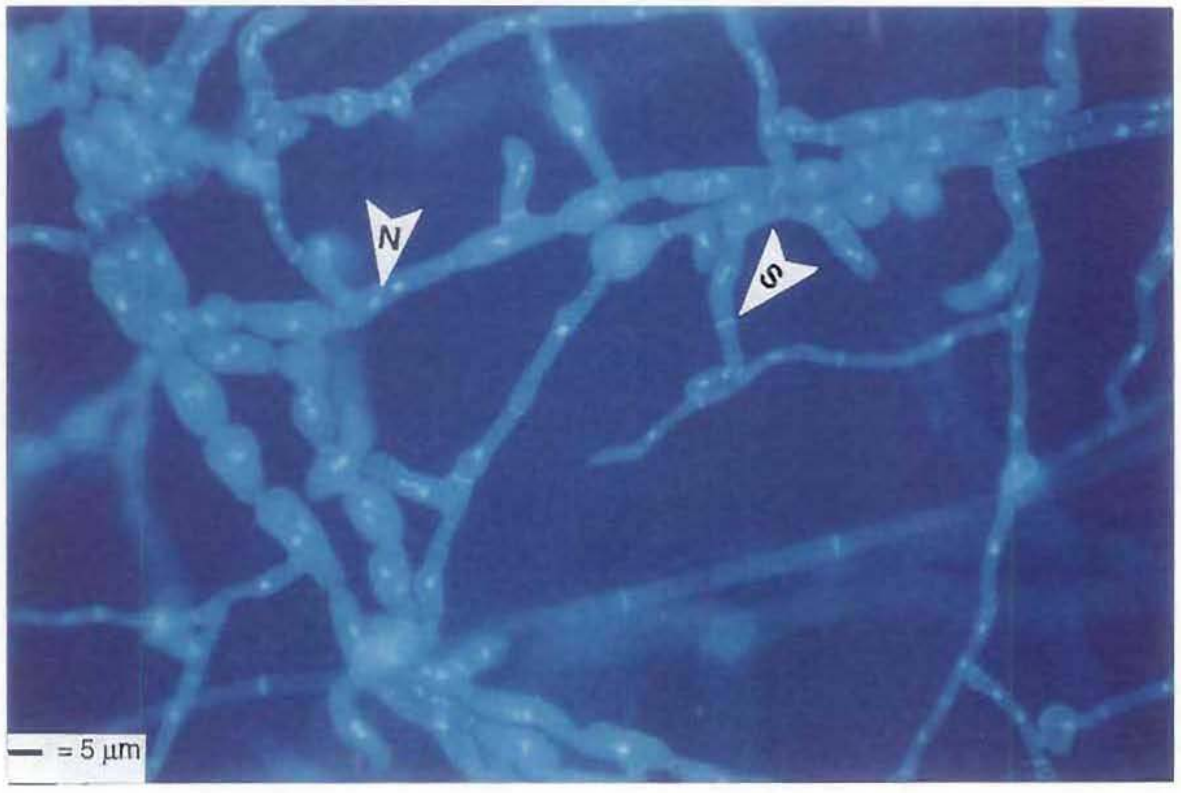


Figure 2.30 Stained nuclei (N) and septa (S) of uni, bi, multinucleate monilioid cells of SDG 6.

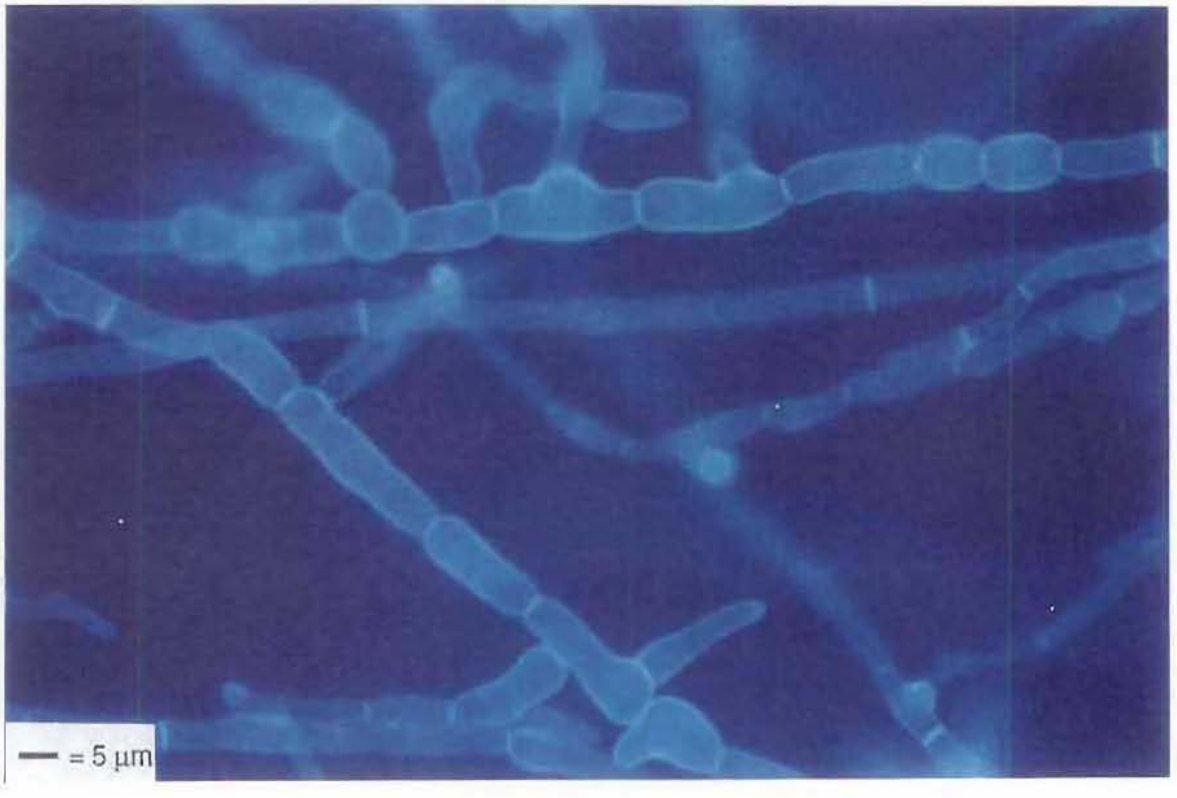


Figure 2.31 Stained septa of beaded oidia-like cells of SHG 3.

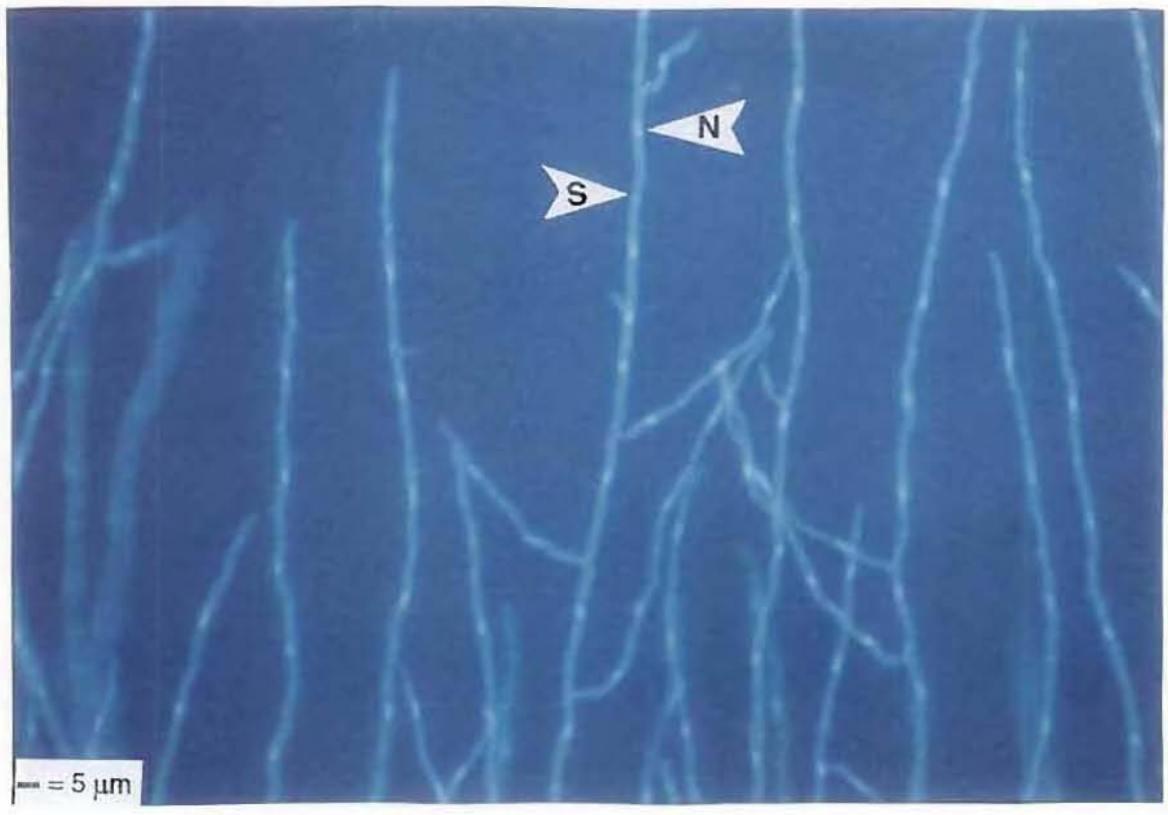


Figure 2.32 Stained nuclei (N) and septa (S) of SDG 7.

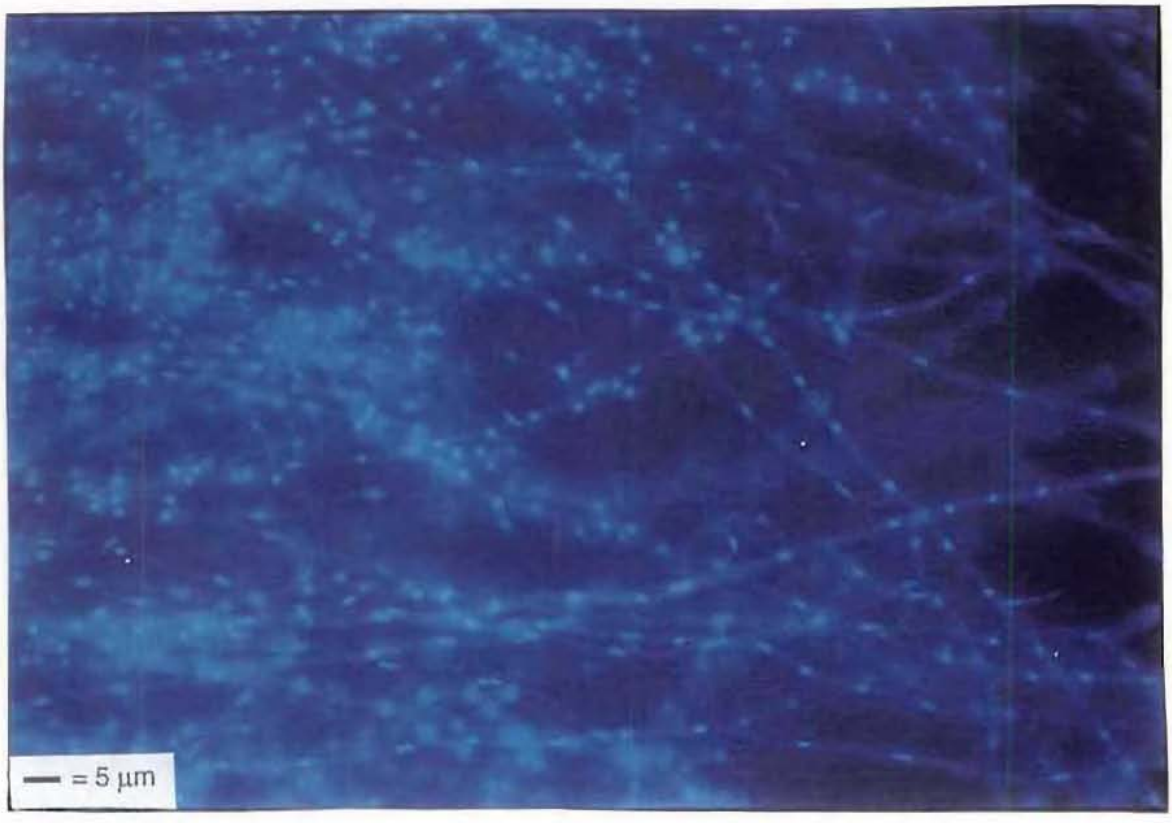


Figure 2.33 Stained nuclei (N) of uniuncleate cells of SHG 3.

2.3.1 (c) Effect of temperature and pH on growth rates.

Incubation temperature had an effect on the radial growth of all sterile isolates as most isolates had low radial growth at the two extremes of the temperature range (Table 2.7). Apart from SDG 6, isolates did not grow at 35°C, and mean colony growth for all isolates was optimal at 15 - 25°C (Table 2.7).

Sterile dark fungi were the most common fungi associated with roots of plants growing in cold environments such as those encountered in cool temperate, subalpine or even arctic environments (Bissett and Parkinson 1979a, 1979b, Haselwandter and Read 1980, Read and Haselwandter 1981, Fisher *et al.* 1995a). The average soil temperatures in the Waikato region range between 15-20°C so it was not unexpected that these sterile isolates grew best at incubation temperatures of 20°C (Table 2.7).

Table 2.7 Mean colony growth (mm) of sterile fungi inoculated onto PCA and incubated at different temperatures.

Temperature °C	10	15	20	25	30	35
Fungal group						
Sterile dark group 1	43.0 ± 8.1	75.0 ± 6.5	80 +	80 +	70.0 ± 3.0	0
Sterile dark group 3	2.0	4.0 ± 0.5	28.4 ± 4.0	27.1 ± 2.2	20.0 ± 0.5	0
Sterile dark group 5	14.2 ± 5.2	26.1 ± 6.4	51.3 ± 3.5	49.0 ± 6.4	6.2 ± 1.2	0
Sterile dark group 6	42.3 ± 2.6	67.0 ± 7.0	80+	80+	70.0 ± 10	2.0
Sterile dark group 7	8.0 ± 2.0	12.0 ± 4.0	19.0 ± 2.0	18.0 ± 2.2	3.0	0
Sterile dark group 8	5.3 ± 1.3	6.5 ± 1.4	7.2 ± 2.5	6.0 ± 2.0	5.2 ± 0.9	0
Thozetella tocklaiensis	6.2 ± 3.1	10.5 ± 3.0	16.4 ± 0.5	16.0 ± 3.0	10.1 ± 0.9	0
Sterile hyaline group 1	7.0 ± 2.0	9.0 ± 2.0	11.0 ± 2.0	15.4 ± 7.6	3.0	0
Sterile hyaline group 3	3.0	8.0 ± 3.2	13.7 ± 1.6	13.0 ± 2.0	1.0	0

All isolates grew at all pH levels however no sporulation was induced apart from *Thozetella tocklaiensis* which was induced to sporulate at pH 9 (Table 2.8). Results indicate these isolates have a wide tolerance of pH and that colony diameters could not be differentiated between most pH levels. Both sterile hyaline groups 1 and 3 had lower colony growth at high pH. These results suggests the pH of most pastoral soils in New Zealand would be unlikely to influence the distribution of these sterile fungi as they could grow on media over a wide pH range.

Table 2.8 Mean and standard deviation of colony growth (mm) of sterile fungi inoculated onto PCA at different pH levels.

pH:	4	5	6	7	8	9
Fungal group						
Sterile dark group 1	60.0 ± 7.1	66.3 ± 9.0	69.0 ± 8.5	67.0 ± 8.5	66.0 ± 11.0	60.3 ± 14.4
Sterile dark group 3	14.5 ± 0.9	12.5 ± 1.1	11.8 ± 1.8	11.5 ± 1.8	11.5 ± 2.2	11.5 ± 1.5
Sterile dark group 5	42.8 ± 6.3	43.0 ± 7.3	44.3 ± 6.7	46.0 ± 6.1	43.3 ± 7.0	45.5 ± 7.7
Sterile dark group 6	63.3 ± 3.1	68.5 ± 7.7	71.5 ± 7.2	76.3 ± 4.0	75.5 ± 4.9	70.5 ± 7.5
Sterile dark group 7	14.3 ± 0.8	16.0 ± 0.7	21.5 ± 1.1	22.5 ± 2.2	23.8 ± 1.3	15.0 ± 1.0
Sterile dark group 8	16.3 ± 1.9	13.0 ± 0.7	13.3 ± 0.8	13.0 ± 1.2	13.0 ±1.2	9.3 ± 0.8
Sterile hyaline group 1	19.3 ± 0.4	15.3 ± 1.3	13.8 ± 0.4	12.3 ± 1.5	11.8 ± 1.5	9.8 ± 1.1
Sterile hyaline group 3	23.3 ± 0.8	23.0 ± 1.9	21.8 ± 1.6	20.5 ± 2.3	20.0 ± 1.4	13.5 ± 1.1
Thozetella tocklaiensis	34.3 ± 2.6	33.0 ± 0.8	34.0 ± 2.2	34.3 ± 3.1	31.0 ± 2.5	20.7 ± 1.3*

* sporulation induced

2.3.1 (d) Effect of fungicide on growth rates.

Most sterile isolates were sensitive to the fungicide amendment to PCA plates as colony growth was inhibited (Table 2.9). An exception to this were the isolates of sterile dark group 6 which were insensitive to benomyl and grew at all concentrations apart from 0.25 mg/ml (Figure 2.34). The mean radial growth of SDG 6 isolates decreased as the concentration of fungicide increased. Four other fungi; SDG1, *T. tocklaiensis, C. scoparium,* and *F. crookwellense,* were not inhibited at the lowest benomyl concentration, 0.001 mg/ml (Table 2.9). The growth of SDG 6 isolates on benomyl amended media confirmed these isolates as a distinct taxonomic group and could therefore be used in conjunction with cultural characteristics to distinguish this group from other sterile dark isolates.

Table 2.9 Mean and standard deviation of colony growth (mm) of sterile fungi inoculated onto PCA amended with different concentrations of Benomyl.

Benomyl mg/ml	0.25	0.15	0.10	0.05	0.01	0.001	0 (control)
Fungal group							
Sterile dark group 1	0	0	0	0	0	80.8 ± 2.6	> 80
Sterile dark group 3	0	0	0	0	0	0	15.6 ± 1.4
Sterile dark group 5	0	0	0	0	0	0	31.8 ± 2.9
Sterile dark group 6	0	16.4 ± 4.5	35.2 ± 5	42 ± 1.9	58 ± 6.5	77.8 ± 1.7	> 80
Sterile dark group 7	0	0	0	0	0	0	22.0 ± 2.1
Sterile dark group 8	0	0	0	0	0	0	10.6 ± 0.6
Sterile hyaline group 1	0	0	0	0	0	0	10.6 ± 0.8
Sterile hyaline group 3	0	0	0	0	0	0	16.6 ± 1.5
T. tocklaiensis	0	0	0	0	0	32.4 ± 2.9	37.2 ± 1.5
F. crookwellense	0	0	0	0	0	80	> 80
C. scoparium	0	0	0	0	0	80	> 80

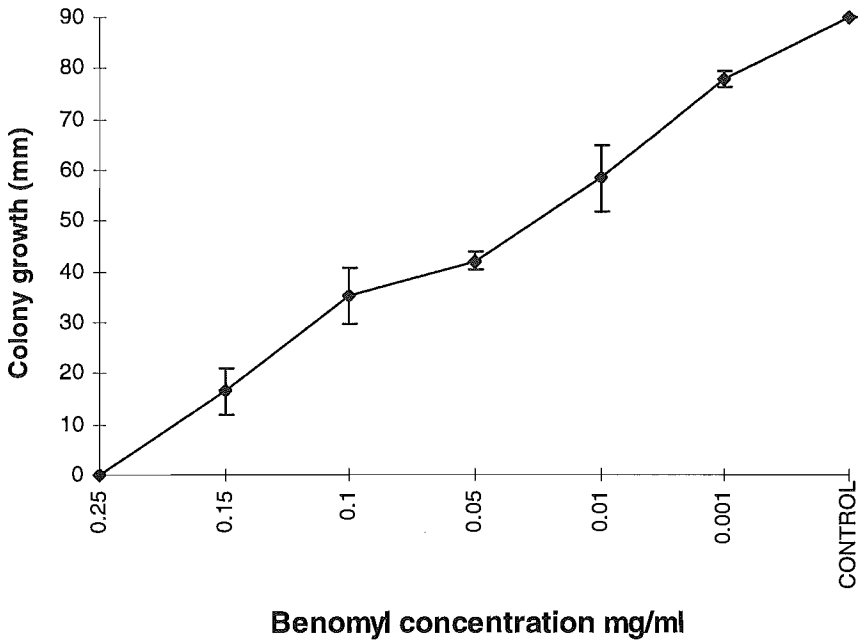


Figure 2.34 Mean colony growth of isolates of sterile dark group 6 on PCA plates amended with different concentrations of benomyl.
Error bars represent standard deviation of the mean.

2.3.1 (e) Assimilation of carbon and nitrogen sources.

Colony growth on single carbon sources ranged from slow with sparse aerial mycelium to rapid with dense aerial mycelium (Table 2.10, Figure 2.35). There was growth on all compounds tested although it was limited on some compounds and in some groups, particularly SDG 6 on fructose, melizitose and raffinose (Table 2.10). Mean radial growth of many sterile isolates on succinic acid plates was higher than most other carbon compounds (Figures 2.36-2.44), and the growth on the remaining compounds was similar for most sterile isolates.

Table 2.10 Mycelial growth of sterile fungi on carbon compounds.

	SDG 1	SDG 3	SDG 5	SDG 6	SDG 7	SDG 8	SHG 1	SHG 3	TT
Arabinose	+++	+++	++	+++	+++	+++	+++	++	+++[S]
Cellobiose	+++	+++	++	++	++	++	+++	++	+++
Fructose	+++	+++	++	+	++	++	+++	++	+++[S]
Galactose	++	++	++	+++	+	++	++	++	+++
Glucose	+++	+++	+++	+++	+++	+++	+++	+++	+++
Inositol	++	+++	++	++	++[C]	++	+++	++	+++[S]
Lactose	++	++	++	+++	++	++	+++	++	+++
Maltose	+++	+++	+++	+++	+++	++	+++	++	+++
Mannitol	++	+++	++	+++	++[C]	++	++	++	+++[S]
Mannose	+++	+++	++	+++	+++	++	++	+++	+++
Melizitose	+++	+++	++	+	++[C]	++	++	++	+++
Raffinose	+++	+++	++	+	++	++	+++	+++	+++[S]
Succinic acid	++	+++	+++	+++	+++	+++	+++	+++	+++[S]
Trehalose	+++	+++	++	++	++	++	+++	++	+++
Xylose	+++	++	+++	+++	+++	++	+++	++	+++[S]

+++ = rapid dense mycelial growth after 7 days incubation, ++ = sparse mycelial growth after 7 days incubation, + sparse mycelial growth after 14 days incubation, - = no growth after 14 days incubation., [S] = sporulation induced [C] = production of chlamydospores/ monilioid hyphae induced. TT = *T. tocklaiensis.*

Colony growth on single nitrogen sources also ranged from slow with sparse aerial mycelium to rapid with dense aerial mycelium (Table 2.11, Figure 2.35). Most isolates were able to assimilate all tested nitrogen compounds. However exceptions were observed, urea was not assimilated by two sterile groups (Table 2.11), growth of SDG 5 was not supported by trytophan, which also restricted growth of SDG 1, SDG 3, and SDG 6. Isolates of *T. tocklaiensis* could not assimilate methionine. Mean radial growth of sterile isolates measured on nitrogen compounds was more variable (Figures 2.36 - 2.44). There were significantly different ($P<0.01$) mean growth rates measured between the different carbon and nitrogen compounds for each sterile group (Figures 2.36 - 2.44).

Sterile mycelium of *T. tocklaiensis* was induced to produce sporodochia on some of the carbon and nitrogen assimilation plates (Tables 2.10, 2.11), which could be used as an additional method to trigger sporulation of this fungus. Sporulation of other sterile isolates was not induced by any of these compounds. However, production of chlamydospore-like monilioid cells by all SDG 1 isolates was induced on arginine (Table 2.11, Figures 2.45, 2.46), structures previously absent on other growth media, and is further evidence that these isolates are a distinct taxonomic group.

Table 2.11 Mycelial growth of sterile fungi on Nitrogen compounds.

	SDG 1	SDG 3	SDG 5	SDG 6	SDG 7	SDG 8	SHG 1	SHG 3	TT
Arginine	+++[C]	++	+++	+++	++	++	+++	++	+++
Asparagine	+++	++	++	+++[C]	++	++	+++	++	+++
Cysteine	++	+	++	++	++	++	+++	++	++
Glutamine	+++	++	+++	+++	++	++	+++	++	+++
Histidine	+++	+++	+++	+++	+++	+++	+++	+++	+++
Leucine	+++	+++	+++	+++	+++	+++	+++	++	+++[S]
Methionine	+++	+++	+++	+++	+++	+++	++	+++	-
Trytophan	++	++	-	+	+++	+++	+++	+++	+++
NH_4Cl	+++	+++	++	+++	+++	++	+++	+++	+++
$NH_4(NO_3)$	+++	++	++	+++	++	++	+++	+++	+++[S]
NH_4SO_4	+++	+++	+++	+++	+++	+++	+++	+++	+++
KNO_3	+++	+++	+++	+++	+++	+++	+++	+++	+++[S]
KNO_2	+++	+++	+++	+++	+++	+++	+++	+++	+++
$NaNO_3$	+++	+++	++	+++	+++	+++	+++	+++	+++
$NaNO_2$	+++	+++	+++	+++	+++	+++	+++	+++	+++
Urea	+++	+	+	++[C]	+	-	-	+++	+

++ = rapid dense mycelial growth after 7 days incubation, ++ = sparse mycelial growth after 7 days incubation, + sparse mycelial growth after 14 days incubation, - = no growth after 14 days incubation, [S] = sporulation induced or production of chlamydospores, [C] = production of chlamydospores/ monilioid hyphae induced. TT = *T. tocklaiensis,* FC = *Fusarium crookwellense*

Ross (1960) showed that sterile fungi isolated from tussock-grassland soils in New Zealand were able to utilise a wide range of carbon and nitrogen compounds. These results were consistent with the observations obtained in this study and also indicates that all isolates possessed the ability to synthesise many different enzymes. However, Ross (1960) also reported sterile fungi were more discriminating between different carbon compounds than other soil fungi that were tested such as *Cylindrocarpon* and *Fusarium.*

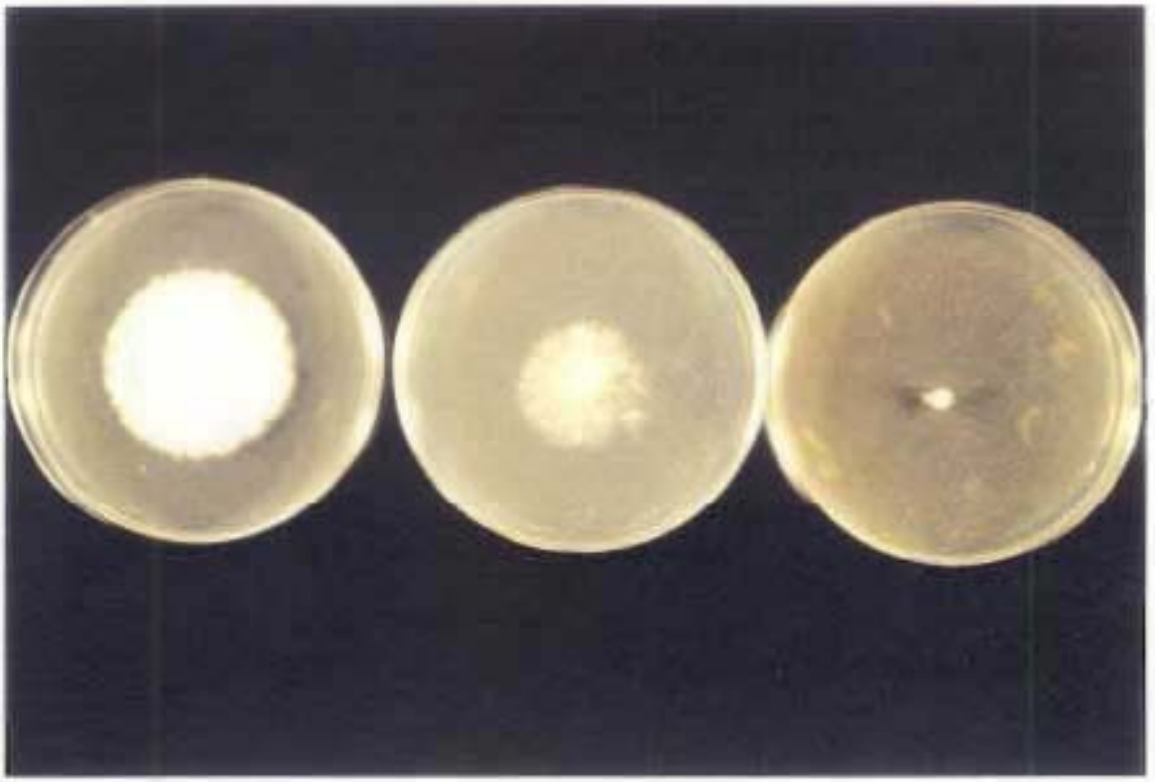


Figure 2.35 Colony growth observed on Carbon and Nitrogen assimilation plates after 14 days, +++ dense mycelial growth, ++/+ sparse mycelial growth, - no growth.

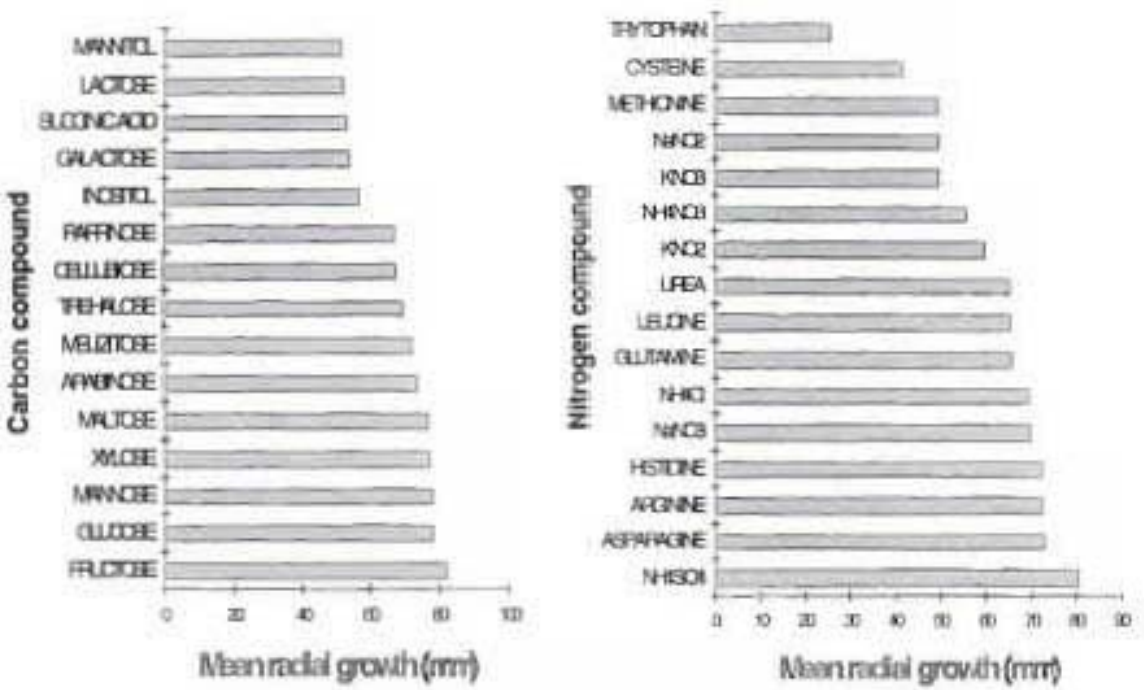


Figure 2.36 Colony diameters of sterile dark group 1 on carbon and nitrogen assimilation plates. Carbon SED = 7.910; Nitrogen SED = 6.090

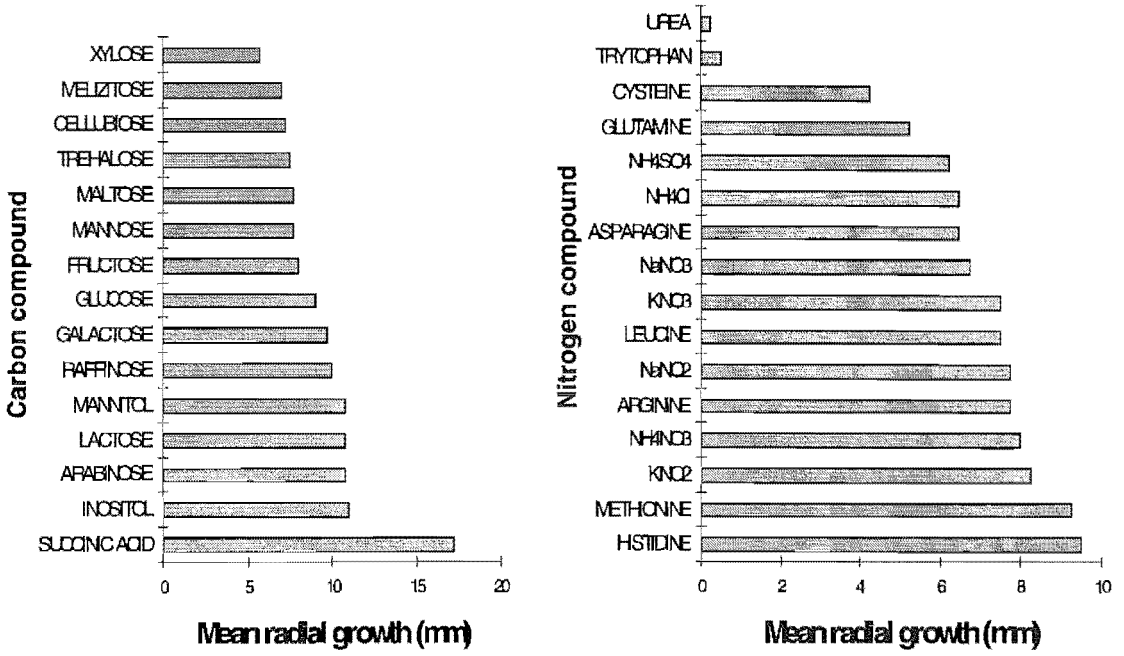


Figure 2.37 Colony diameters of sterile dark group 3 on carbon and nitrogen assimilation plate. Carbon SED = 0.921; Nitrogen SED = 1.030

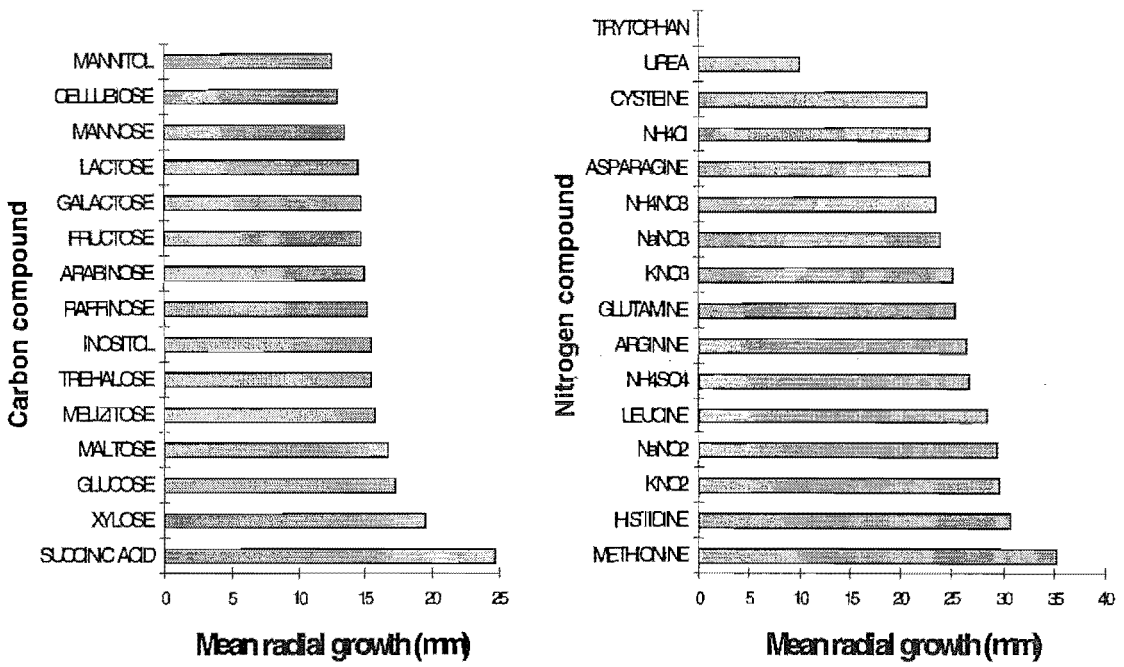


Figure 2.38 Colony diameters of sterile dark group 5 on carbon and nitrogen assimilation plates. Carbon SED = 1.798; Nitrogen SED = 3.498

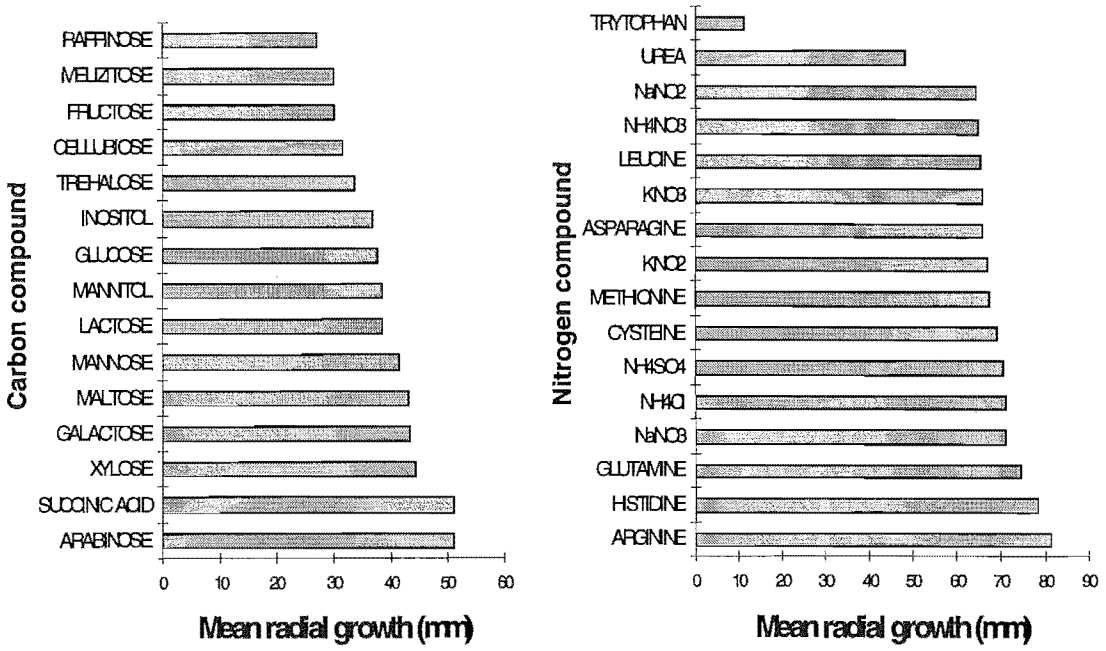


Figure 2.39 Colony diameters of sterile dark group 6 on carbon and nitrogen assimilation plates. Carbon SED = 4.768; Nitrogen SED = 5.281

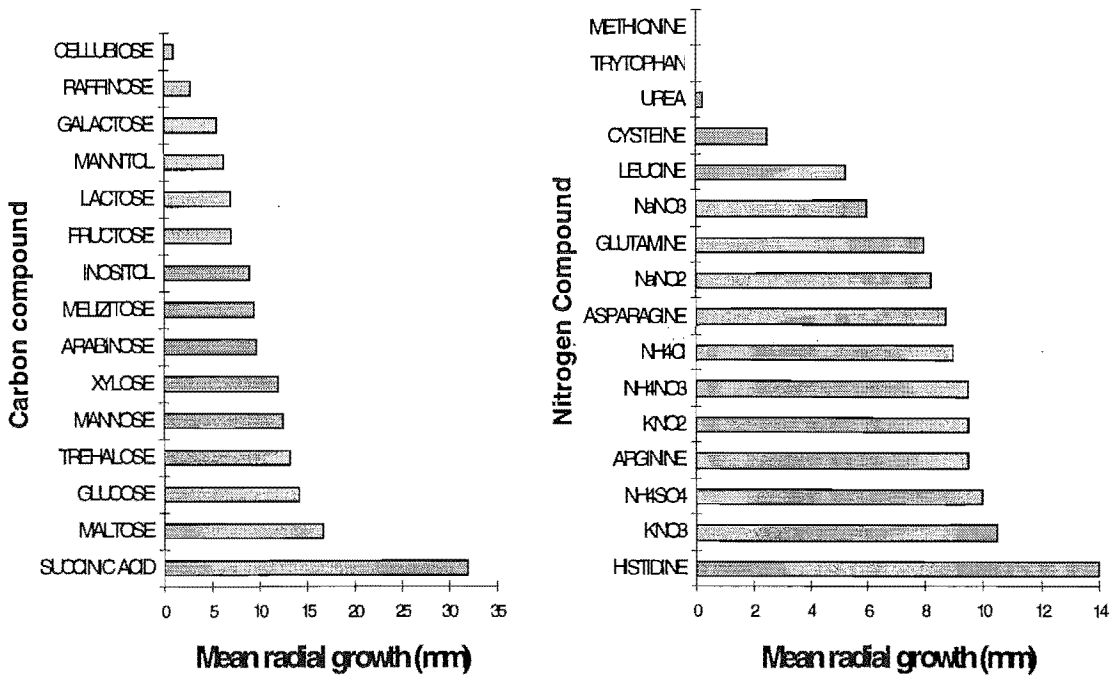


Figure 2.40 Colony diameters of sterile dark group 7 on carbon and nitrogen assimilation plates. Carbon SED = 2.175; Nitrogen SED = 1.668

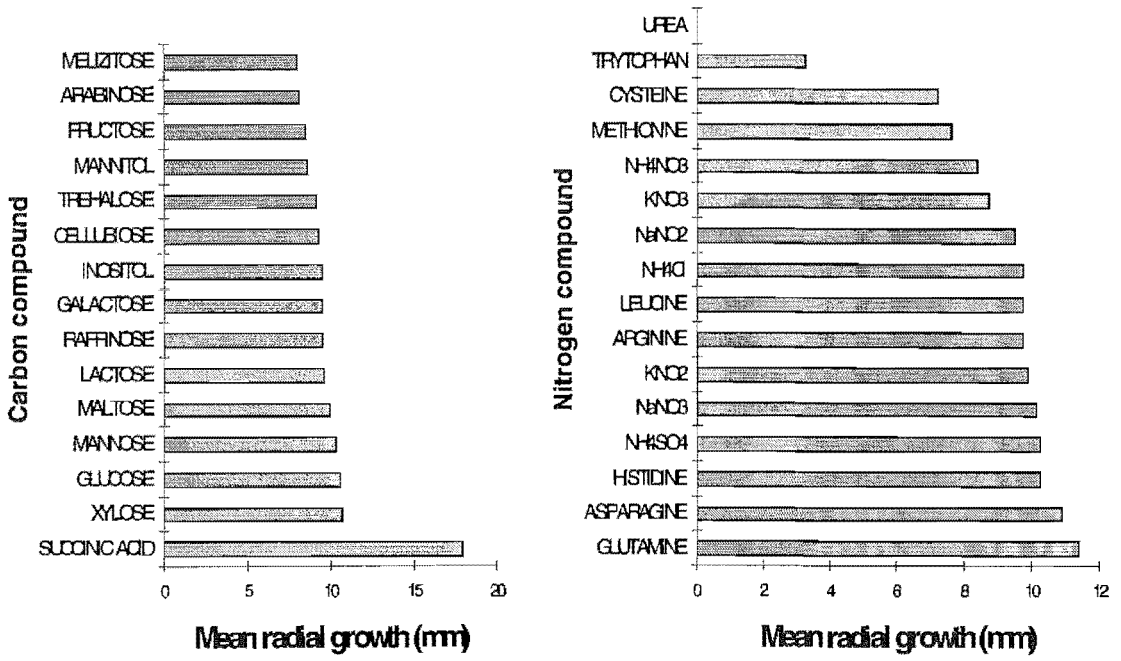


Figure 2.41 Colony diameters of sterile dark group 8 on carbon and nitrogen assimilation plates. Carbon SED = 1.237; Nitrogen SED = 0.978

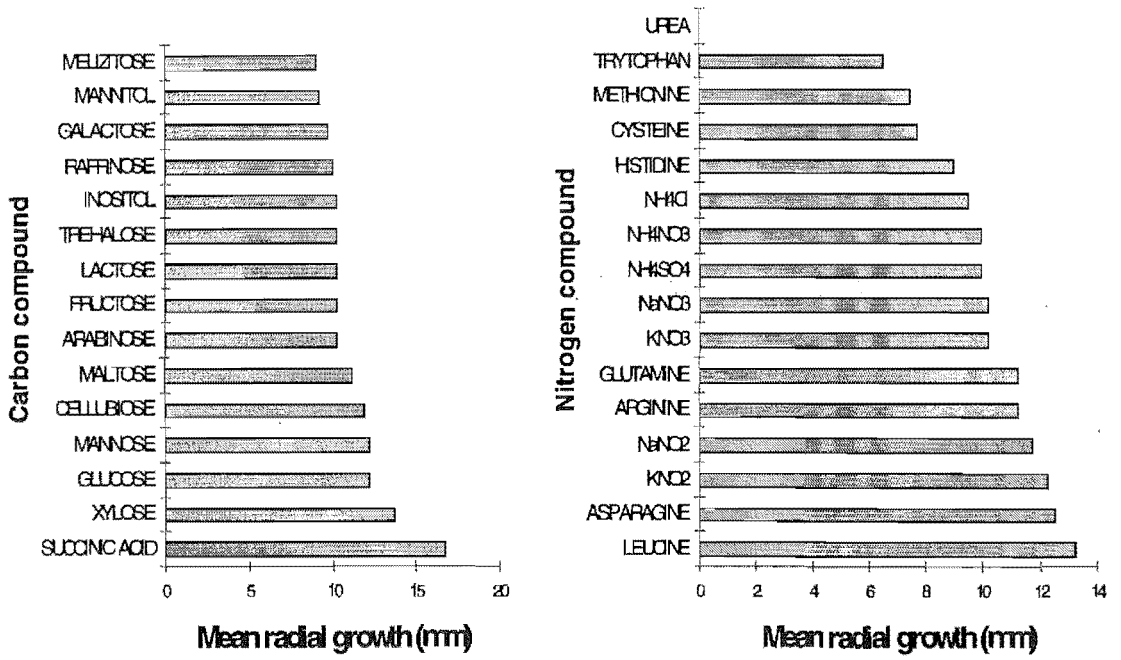


Figure 2.42 Colony diameters of sterile hyaline group 1 on carbon and nitrogen assimilation plates. Carbon SED = 1.207; Nitrogen SED 1.363

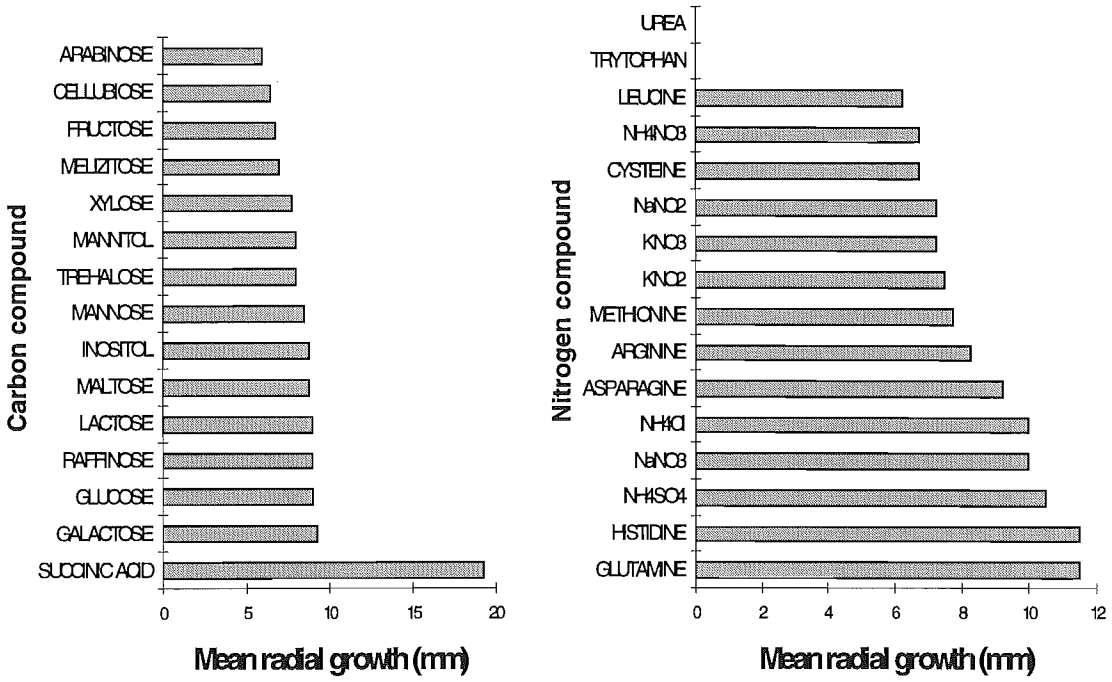


Figure 2.43 Colony diameters of sterile hyaline group 3 on carbon and nitrogen assimilation plates. Carbon SED = 0.634; Nitrogen SED = 1.012

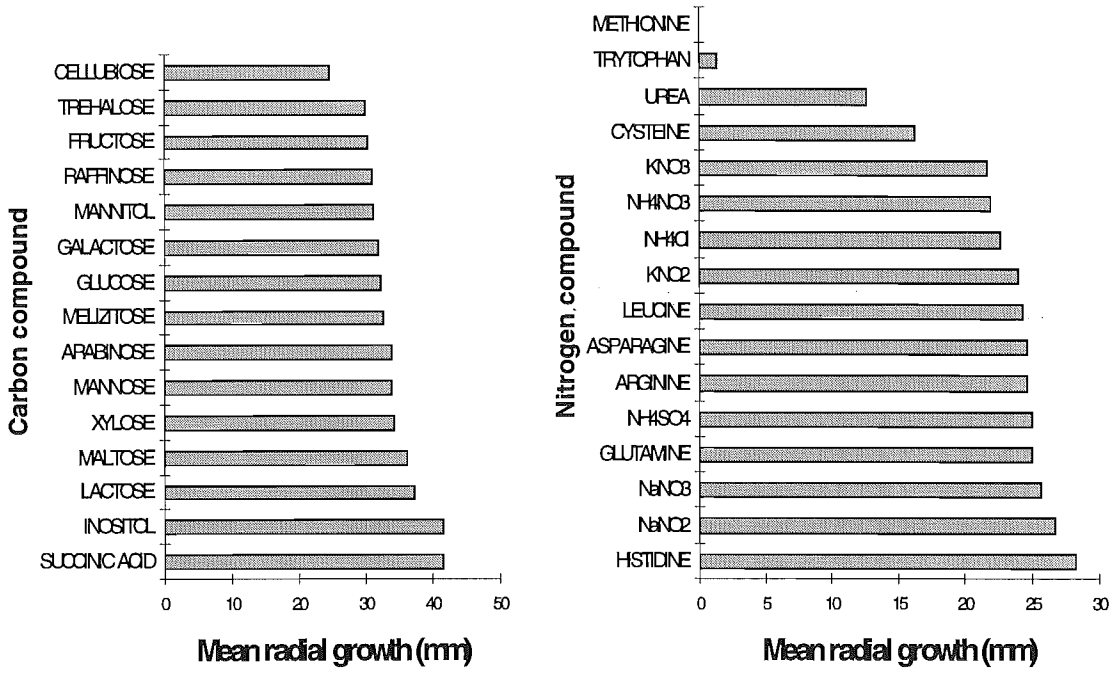


Figure 2.44 Colony diameters of *Thozetella tocklaiensis* on carbon and nitrogen assimilation plates. Carbon SED = 2.575; Nitrogen SED = 1.203

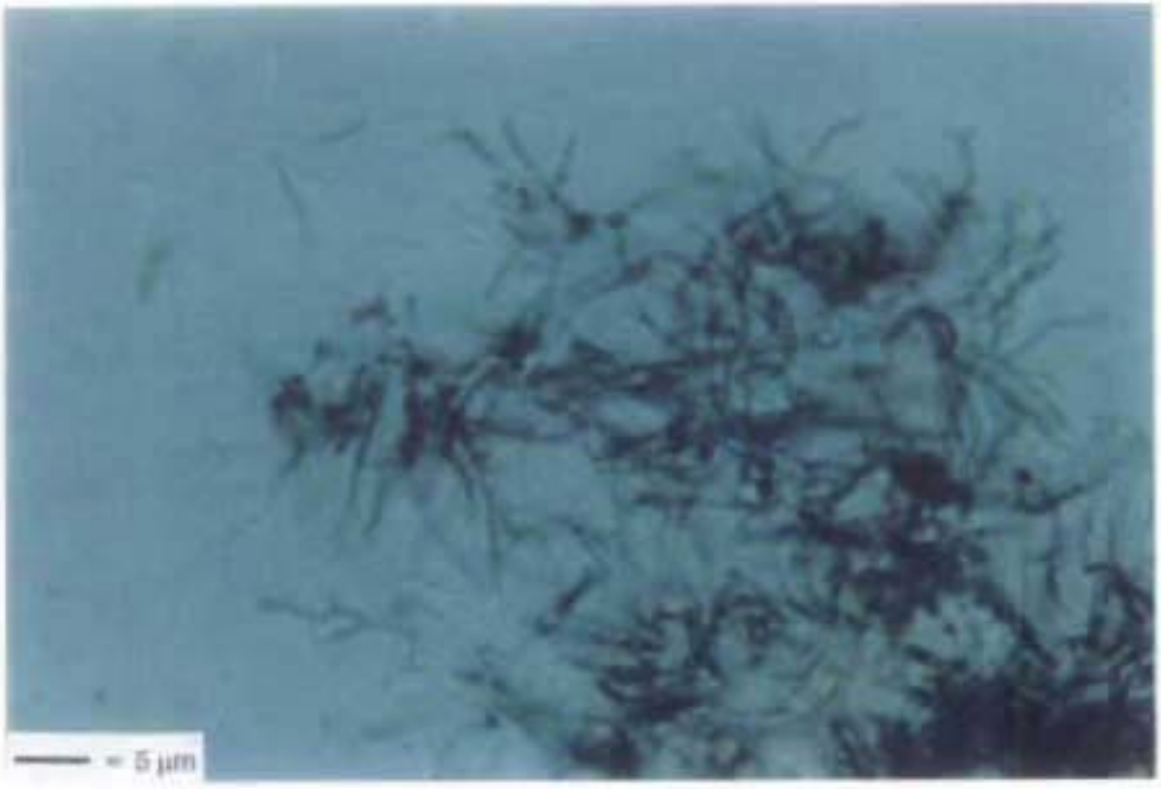


Figure 2.45 A tuft of chlamydospore-like monilioid cells by SDG 1 produced on arginine plates (200x).

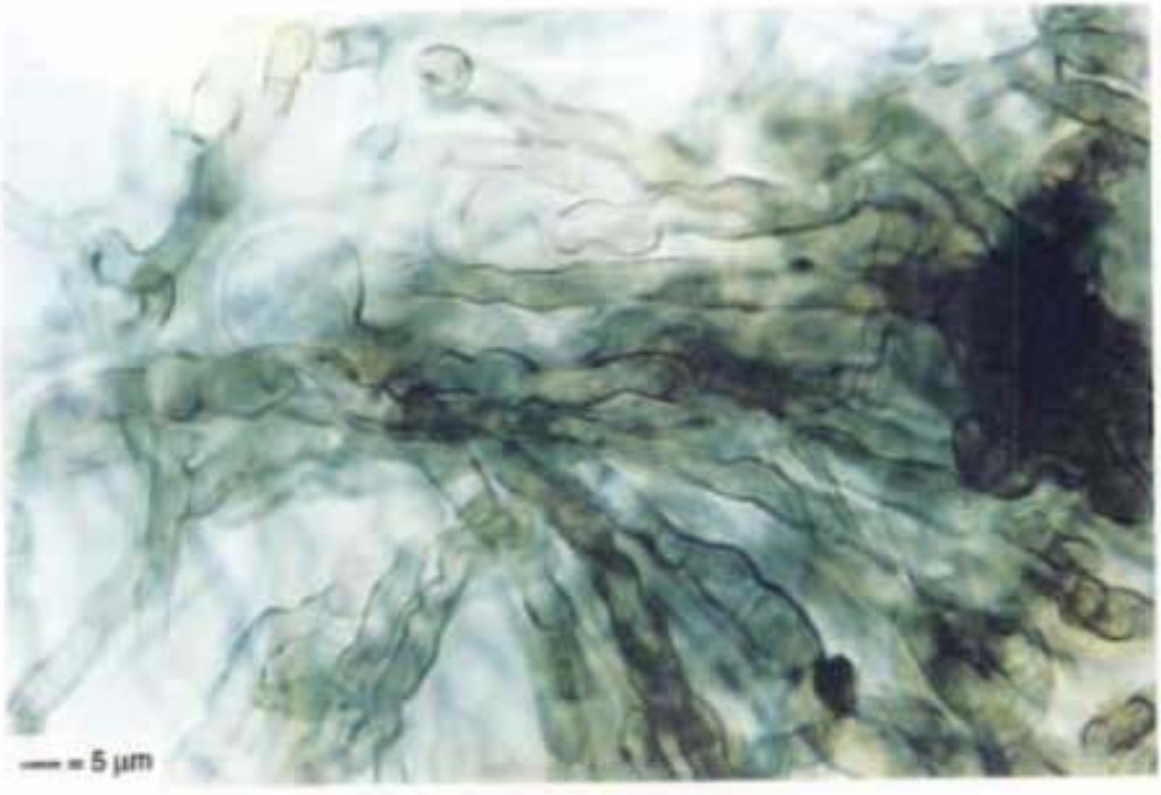


Figure 2.46 Chlamydospore-like monilioid cells by SDG 1 produced on arginine plates (400x).

2.3.1 (g) Inoculation of sterile fungi onto axenically grown seedlings.

No sporulation was induced by inoculating seedlings. The majority of sterile isolates were either weakly or non pathogenic to the pasture seedlings tested (Table 2.12, Figure 2.47), and few disease symptoms were observed on most grass seedlings. Light to dark brown discoloration was more commonly observed on inoculated legume seedlings but this did not affect their overall growth. Sterile hyaline groups 1 and 2 were mildly pathogenic to most hosts as brown root discoloration and some lesions were observed on roots. Sterile dark group 4 and sterile hyaline group 2 were the only isolates that were pathogenic, as lesions and necrosis were observed on red and white clover seedlings, ryegrass and browntop (Table 2.12).

Table 2.12 Sterile fungi classed as pathogenic to pasture legume and grass seedlings using a Petri plate technique.

Fungal group	PATHOGENICITY											
	RC*	**WC**	**SC**	**RG**	**YF**	**CF**	**LO**	**SV**	**BT**	**TI**	**SB**	**TF**
Sterile Dark Group 1	1	2	1	2	0	0	1	0	0	1	0	0
Sterile Dark Group 2	1	1	1	0	0	0	1	0	1	0	0	0
Sterile Dark Group 3	0	0	0	0	0	0	0	0	0	0	0	0
Sterile Dark Group 4	3	2	2	1	0	0	0	1	0	0	0	0
Sterile Dark Group 5	2	1	1	0	0	1	1	0	0	0	0	0
Sterile Dark Group 6	2	1	1	2	1	1	1	1	1	1	0	0
Sterile Dark Group 7	0	0	0	0	0	0	0	0	0	0	0	0
Sterile Dark Group 8	0	1	0	0	0	0	0	0	0	0	0	0
Sterile Dark Group 9	0	0	0	0	0	0	0	0	0	0	0	0
Sterile Hyaline Group 1	2	2	0	1	0	2	1	2	2	1	0	1
Sterile Hyaline Group 2	3	3	2	3	2	1	2	2	3	1	0	0
Sterile Hyaline Group 3	0	0	0	0	0	0	0	0	0	1	0	0
Control	0	0	0	0	0	0	0	0	0	0	0	0

* **RC** red clover, **WC** white clover, **SC** subterranean clover, **RG** ryegrass, **YF** Yorkshire fog, **CF** cocksfoot, **LO** lotus, **SV** sweet vernal, **BT** browntop, **TI** timothy, **SB** soft brome, **TF** tall fescue.
** **3** pathogenic (average disease scores 4-5), **2** mildly pathogenic (average disease scores 3 -3.9), **1** weakly pathogenic (average disease scores 2- 2.9), **0** non pathogenic (average disease scores 0 - 1.9).

All fungi were observed to invade the root tissues of seedlings apart from SDG 9 where no hyphal colonisation was observed in stained segments. Hyphae of most isolates were observed in the cortex and epidermis tissue of inoculated hosts (Table 2.13, Figures 2.48, 2.49), which confirmed these sterile isolates as root-colonising fungi. Although no sporulation was observed in infected root tissues, three sterile fungi (SDG 6, SHG 1,3) produced papillae, swollen cells and monilioid hyphae in root cells (Figures 2.50 -2.52). These swollen cells particularly those produced by SDG 6 and SHG 3 resembled the oidia produced by root endophytes as described by (Nicolson 1959). While most isolates were restricted to the cortex, hyphae of SDG 6 were observed in the conductive regions of subterranean clover, white clover and browntop, and SHG 1 was also observed to systemically invade cocksfoot seedlings (Table 2.13, Figure 2.52). Waid (1957) reported that dark sterile isolates generally penetrated ryegrass roots more systemically (i.e. xylem vessels and endodermis), than hyaline isolates which colonised surface and outer cortex

regions, however this difference was not observed here as both types penetrated roots to the same degree.

Table 2.13 Colonisation of inoculated seedlings of twelve pasture plants by sterile fungi.

Fungal group	COLONISATION											
	RC*	**WC**	**SC**	**RG**	**YF**	**CF**	**LO**	**SV**	**BT**	**TI**	**SB**	**TF**
Sterile Dark Group 1	2	2	1	1	1	1	2	2	2	2	1	1
Sterile Dark Group 2	0	1	0	0	0	1	0	1	0	1	0	0
Sterile Dark Group 3	1	2	0	0	2	0	2	1	2	1	2	0
Sterile Dark Group 4	1	1	0	0	0	0	0	1	0	1	0	0
Sterile Dark Group 5	2	2	2	2	2	1	1	2	2	1	2	2
Sterile Dark Group 6	2	3	3	2	2	2	2	2	3	2	2	1
Sterile Dark Group 7	0	0	1	0	0	1	1	1	1	0	0	0
Sterile Dark Group 8	0	2	2	1	2	1	1	2	1	1	1	1
Sterile Dark Group 9	0	0	0	0	0	0	0	0	0	0	0	0
Sterile Hyaline Group 1	2	2	2	2	1	3	2	2	2	2	2	1
Sterile Hyaline Group 2	1	1	2	1	0	1	1	1	1	0	0	0
Sterile Hyaline Group 3	1	2	1	1	2	0	2	2	2	1	0	0

* **RC** red clover, **WC** white clover, **SC** subterranean clover, **RG** ryegrass, **YF** Yorkshire fog, **CF** cocksfoot, **LO** lotus, **SV** sweet vernal, **BT** browntop, **TI** timothy, **SB** soft brome, **TF** tall fescue. ** **3** = inner cortex and vascular tissue colonisation, **2** = cortex colonisation, **1** = epidermal colonisation, **0** = hyphal colonisation absent. **NT** = Not tested due to contamination.

Sterile isolates were reisolated on WA from surface sterilised segments and this with the direct observation of hyphae in stained tissues satisfied Koch's postulates that these fungi are true root-colonising fungi. Excluding SHG 2, the remaining sterile groups were mildly pathogenic to non pathogenic. Non-pathogenic sterile fungi have also been previously recorded from grassland soils and plant roots (Morrison *et al.* 1959, Hall 1987).

Hall (1985, 1987) was able to identify a significant proportion of sterile isolates obtained from wheat roots by inoculating isolates onto seedling plates. This method induced many to sporulate and isolates were identified as *Acremonium rutilum, Cylindrocarpon destructans, Phoma leveillei, Rhizoctonia* sp. and *Coprinus* sp. (Hall 1987). Apart from *T. tocklaiensis,* sterile isolates obtained in this study did not sporulate on seedlings and were therefore considered to be truly sterile.

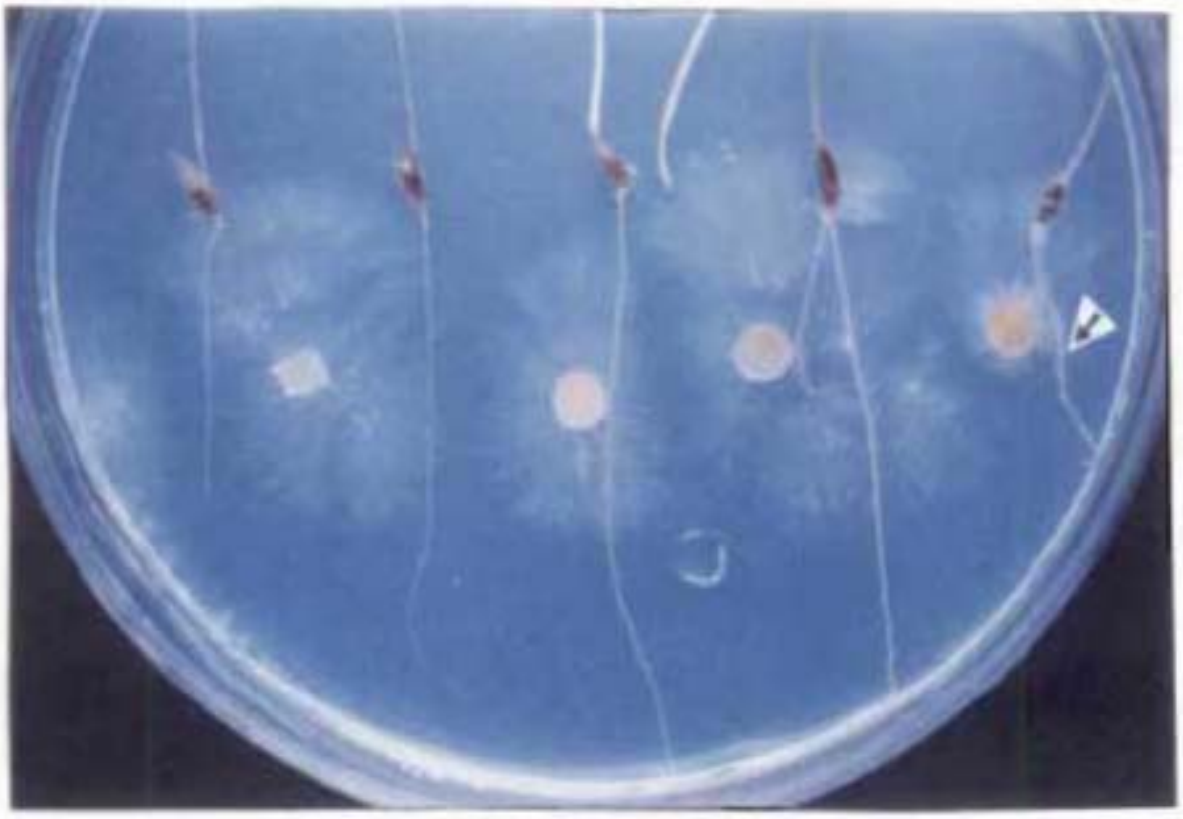


Figure 2.47 Ryegrass seedlings inoculated with an isolate of sterile hyaline group 1 (SHG 1), seedling on far right with discoloration after infection.

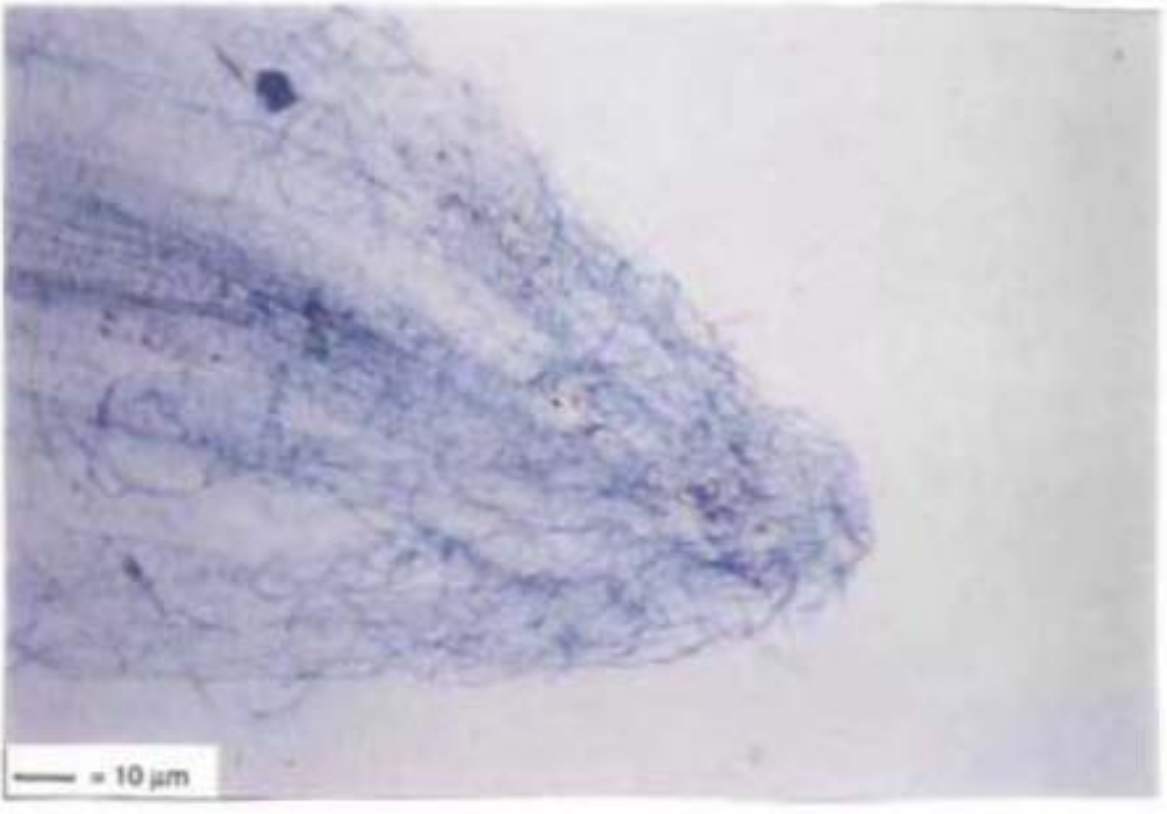


Figure 2.48 Root tip of Lotus colonised by the hyhae of sterile dark isolate 5 (SDG 5).

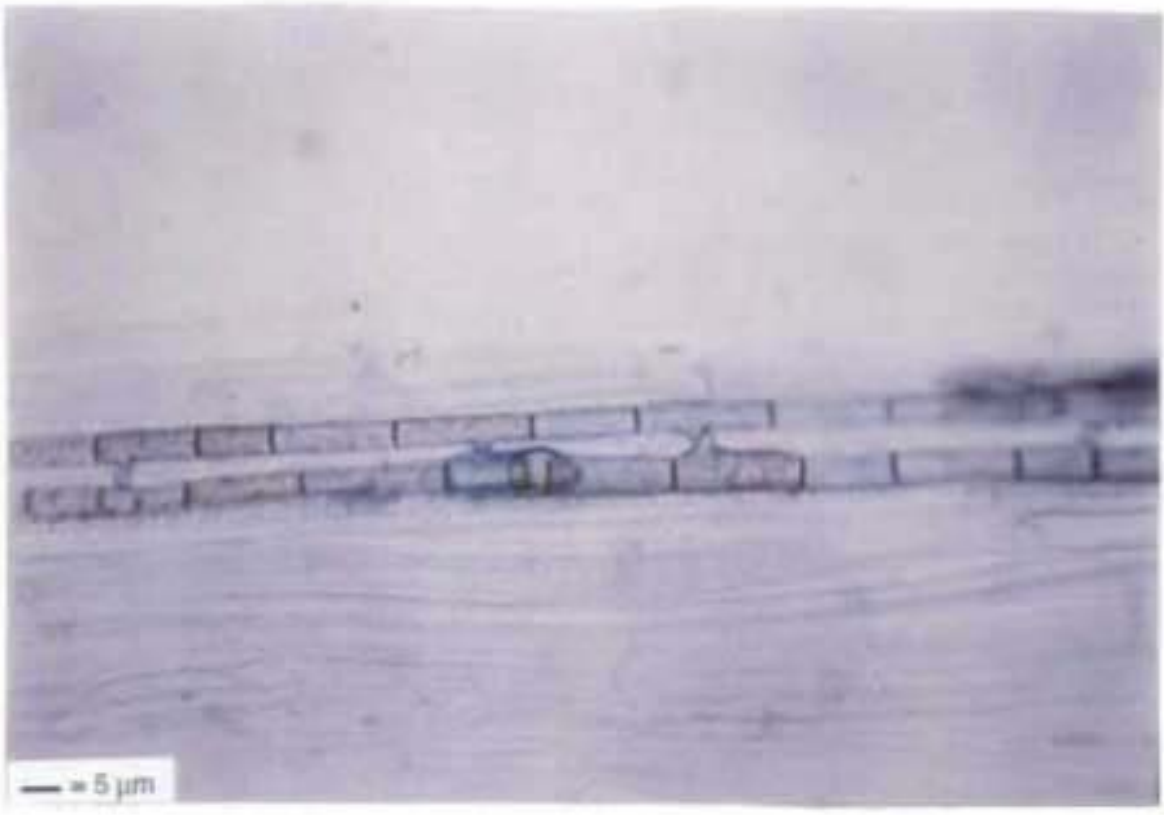


Figure 2.49 Longitudinal section of a white clover root colonised by the hyphae of sterile dark isolate 1 (SDG 1).

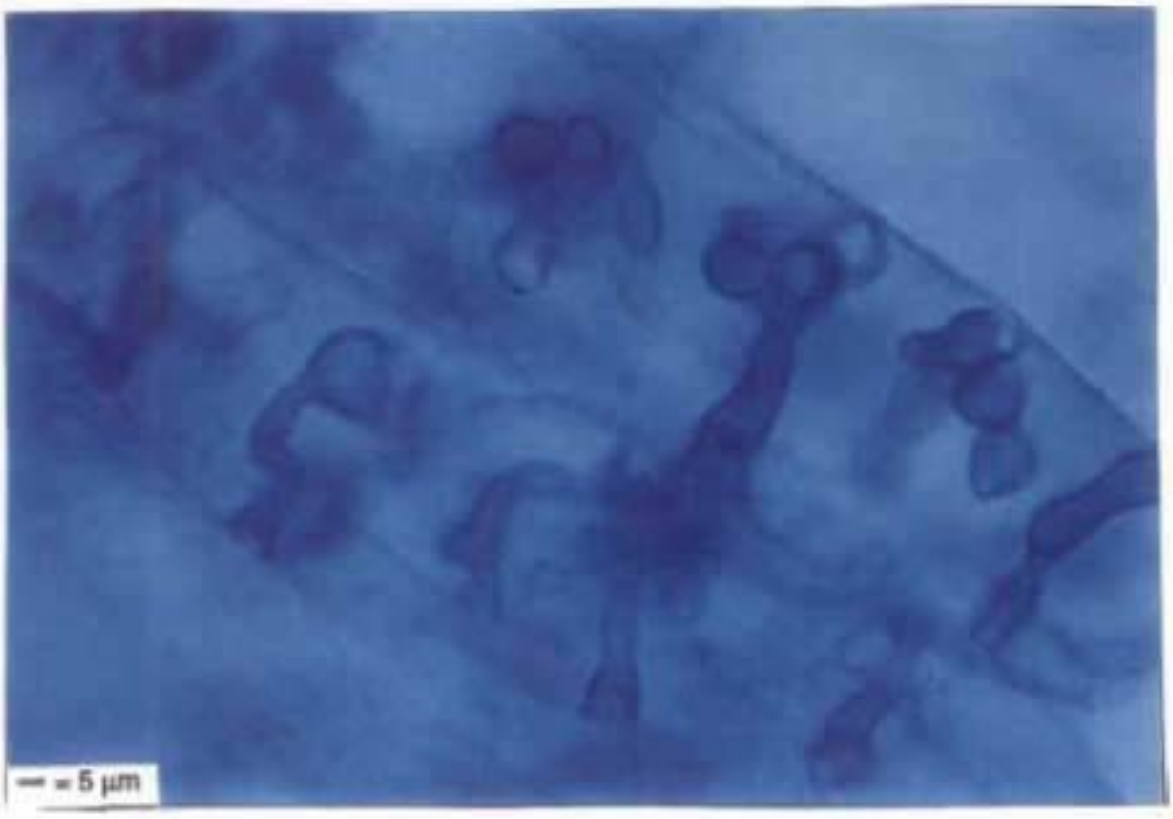


Figure 2.50 Intracellular monilioid hyphae of sterile dark isolate in the cortex of infected ryegrass root.

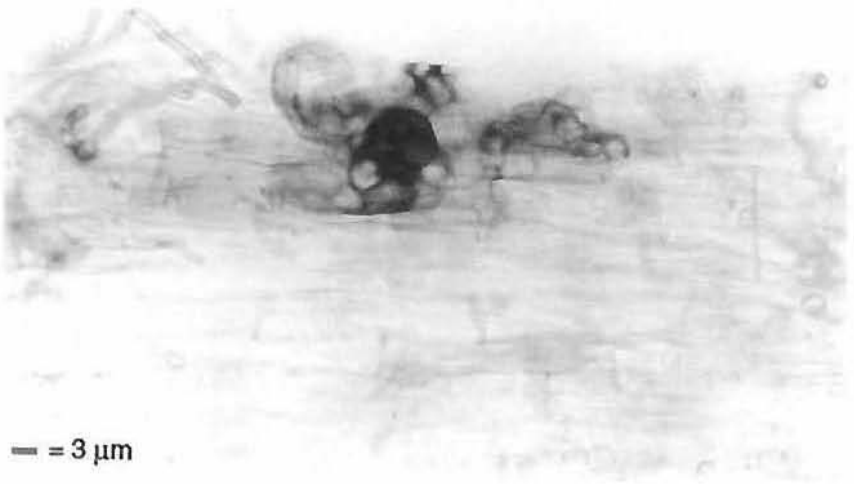


Figure 2.51 Hyphal invasion of a sweet vernal seedling inoculated with sterile hyaline isolate 3 (SHG 3).

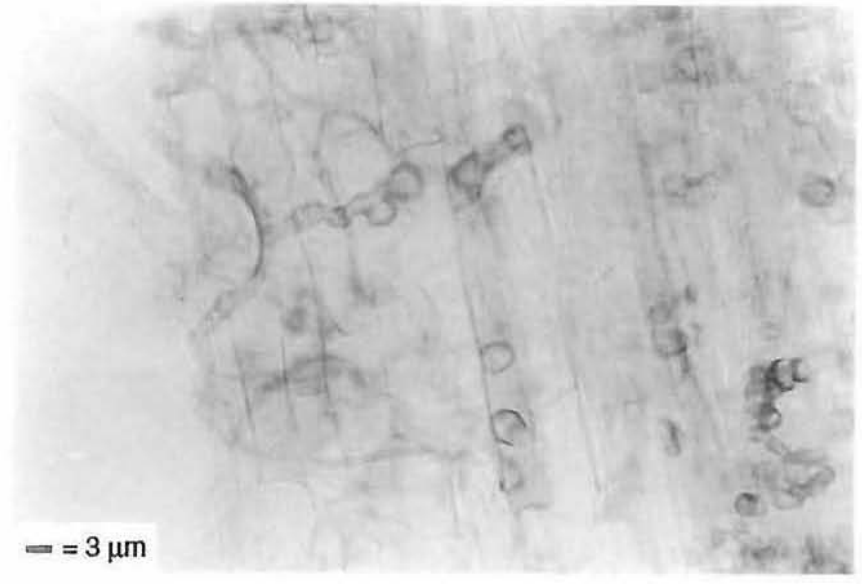


Figure 2.52 Systemic hyphal invasion of a cocksfoot seedling inoculated with sterile hyaline isolate 1 (SHG 1).

2.3.1 (g) Growth of sterile fungi on different substrates. (liquid culture, kibbled wheat, ryegrass seed).

No sterile isolate was induced to sporulate on the three substrates tested, however sterile dark group 8 was found to produce copious dark green exudate in liquid culture. Each method was scored for mycelial growth on each substrate (Table 2.14) and, for the purposes of inoculum production, both V8 liquid culture or kibbled wheat substrates gave better mycelial growth than ryegrass seed.

Table 2.14 Growth of sterile fungi on three substrates.

Sterile Fungal Group	V8 broth medium	Kibbled wheat	Ryegrass seed
Sterile dark group 1	+++	+++	+++
Sterile dark group 3	+	+	+
Sterile dark group 5	+++	++	++
Sterile dark group 6	+++	+++	+++
Sterile dark group 7	+	++	+
Sterile dark group 8	+	+	+
Sterile hyaline group 1	++	++	+
Sterile hyaline group 3	++	++	+

+++ rapid and dense mycelial growth, ++ medium mycelial growth, + slow and sparse mycelial growth

2.3.2 EFFECT OF STERILE FUNGI ON THE YIELD OF SHOOT AND ROOTS OF PASTURE PLANTS.

There were few fungal treatments which affected the shoot or root weights of these pasture plants. Sterile hyaline group 1 increased the mean shoot dry weight of clover, sweet vernal and browntop (Table 2.15) which could suggest this fungus has a beneficial influence on shoot growth. There were five fungal treatments which had a deleterious effect on shoot weight, but there was no pasture species which was particularly sensitive to the inoculation of any sterile group.

Table 2.15 Mean dry shoot weight (g) of plants grown in fumigated soil and inoculated with root-colonising sterile fungi.

Sterile Fungal Group	Ryegrass 1	Ryegrass 2	White clover	Sweet vernal	Browntop	Soft brome
Sterile dark group 1	0.620	0.590	0.317	0.370	0.530	0.563
Sterile dark group 3	0.630	0.553	0.270	0.456	0.437	0.553
Sterile dark group 5	0.630	0.683	0.310	0.436	0.393*	0.523
Sterile dark group 6	0.743+	0.457*	0.287	0.433	0.527	0.423*
Sterile dark group 7	0.660	0.550	0.340	0.447	0.373*	0.510
Sterile dark group 8	0.593	0.693	0.210	0.483+	0.493	0.530
Sterile hyaline group 1	0.610	0.703	0.363+	0.513+	0.603+	0.590
Sterile hyaline group 3	0.663	0.623	0.297	0.363	0.513	0.440*
CONTROL	0.655	0.629	0.299	0.385	0.467	0.527
S.E.D	0.105	0.101	0.059	0.074	0.076	0.064

1 = Ryegrass endophyte free Nui, 2 = Ryegrass endophyte infected Nui.
*** denotes a significant difference (P< 0.05) from the control, SED = standard error of deviation from the mean, + denotes a significant increase in weight compared to the controls (P< 0.05)**

Nine sterile dark groups were found to decrease the root weights of inoculated plants (Table 2.16), but as with the shoot weight results, there was no pasture species or fungus which were consistently reduced weights in all treatments. Browntop appeared to be the most susceptible pasture species as four of the treatments reduced browntop root weights. Six fungal treatments were also found to increase the root weight yields.

Table 2.16 Mean dry root weight (g) of plants grown in fumigated soil and inoculated with root-colonising sterile fungi.

Sterile Fungal Group	Ryegrass 1	Ryegrass 2	White clover	Sweet vernal	Browntop	Soft brome
Sterile dark group 1	0.370	0.463	0.163	0.220	0.280	0.343
Sterile dark group 3	0.447	0.277*	0.117	0.263	0.230*	0.523
Sterile dark group 5	0.340	0.460	0.103*	0.217	0.167*	0.347
Sterile dark group 6	0.677+	0.393	0.090*	0.167*	0.250*	0.437
Sterile dark group 7	0.467	0.343	0.153	0.300+	0.283	0.440
Sterile dark group 8	0.400	0.640+	0.083*	0.257	0.237*	0.483
Sterile hyaline group 1	0.493	0.607+	0.130	0.237	0.340	0.553+
Sterile hyaline group 3	0.647+	0.433	0.113	0.237	0.353	0.370
CONTROL	0.407	0.447	0.173	0.228	0.363	0.410
S.E.D	0.110	0.111	0.050	0.057	0.103	0.113

1 = Ryegrass endophyte free Nui, 2 = Ryegrass endophyte infected Nui.
*** denotes a significant difference (P< 0.05) from the control,**
+ denotes a significant increase in weight compared to the controls (P< 0.05)

Few disease symptoms were observed on the roots of inoculated plants, although significant root discoloration was observed on plants inoculated with SDG 5 and SDG 6 (Table 2.17). Overall most of these sterile fungi were not pathogenic to the tested plants, but some had deleterious or beneficial effects on plant yields.

Table 2.17 Mean root disease scores of plants grown in fumigated soil and inoculated with root-colonising sterile fungi.

Sterile Fungal Group	Ryegrass 1	Ryegrass 2	White clover	Sweet vernal	Browntop	Soft brome
Sterile dark group 1	0	0.33	0	0	0	0.33
Sterile dark group 3	1.0*	0.33	0	0	0.67*	0
Sterile dark group 5	1.0*	1.67*	0	0.67*	1.67*	0
Sterile dark group 6	1.0*	1.33*	1.0*	1.33*	1.33*	1.33*
Sterile dark group 7	0	0.33	0	0	0.33	0
Sterile dark group 8	0	0.33	0	0	0	0.33
Sterile hyaline group 1	0	0.67*	0	0	0	0
Sterile hyaline group 3	0.67*	0	0	0	0.33	0
CONTROL	0	0	0	0	0	0
S.E.D	0.427	0.504	0.408	0.44	0.432	0.496

1 = Ryegrass endophyte free Nui, 2 = Ryegrass endophyte infected Nui.
*** denotes a significant difference (P< 0.05) from the control,**
+ denotes a significant increase in weight compared to the controls (P< 0.05)

2.3.3 ASSESSMENT OF ISOZYME ANALYSIS METHODS TO DIFFERENTIATE STERILE GROUPS.

This initial screen revealed a number of isozyme systems that were suitable for further study (Table 2.18). There was little difference in the activity observed between freeze-dried and live mycelium, apart from the PH buffer system where freeze-dried samples had more activity. The Poulik buffer system produced little activity and is probably not suitable for further use. There were 12 enzyme stains that produced visible bands on the five buffer systems screened with PGM the only enzyme stain that produced activity across all buffer systems (Table 2.18).

Table 2.18 The resolution band activity of isozyme and buffer systems tested on freeze dried and live mycelial extracts of five groups of sterile fungi.

Buffer System:	AC		RW		PK		PH		TC	
Enzyme stain	F	M	F	M	F	M	F	M	F	M
AAT	-	-	-	-	-	-	*	-	-	-
AK	+	+	-	-	-	-	*	*	-	-
CK	+	-	-	-	-	-	*	-	-	-
EST-A	-	-	+	+	*	*	*	*	*	*
GDH	-	-	-	-	-	-	+	-	+	+
GPD	-	-	-	-	-	-	-	-	-	-
GPI	-	-	+	+	-	-	-	-	+	+
G6P	+	+	+	*	-	-	+	+	+	+
GUS	-	-	*	*	-	-	-	-	-	-
ICD	-	-	-	-	-	-	+	+	+	+
LAP	-	-	-	-	-	-	*	*	+	+
LDH	-	-	-	-	-	-	-	-	-	-
MDH	+	+	-	-	-	-	-	-	-	-
MPI	-	-	-	-	-	-	-	-	-	-
PEP	-	-	+	+	-	-	-	-	-	-
6PG	+	+	*	*	-	-	-	-	-	-
PGI	+	+	+	+	-	-	+	+	-	-
PGM	+	+	+	+	*	*	+	+	+	+
Total activity	**7**	**6**	**7**	**7**	**2**	**2**	**8**	**5**	**7**	**7**

F = freeze dried mycelium, M = live mycelium,
+ = activity * = slight activity (bands very faint) - = no activity

Some of the buffer and enzyme systems used in this isozyme screen separated isolates into sterile groups by producing similar band patterns after electrophoresis. These systems are summarised below:

(i) Sterile Dark Group 3

Six enzyme stains used on four buffer systems produced similar bands for the three isolates from this sterile group (Table 2.19, Figures 2.53-2.58)

Table 2.19 Isozyme systems which grouped isolates of SDG 3

Buffer System	Enzyme system	see Figure
AC	GPI; MDH	2.53-54
RW	G6P; GPI; EST-A; PGM	2.55-57
PH	G6P; CPK	2.58,59
TC	G6P	2.58

(ii) Sterile Dark Group 5

Five enzyme stains used on two buffer systems produced similar bands for the three isolates from this sterile group (Table 2.20, Figures 2.53-2.62)

Table 2.20 Isozyme systems which grouped isolates of SDG 5

Buffer System	Enzyme system	see Figure
AC	CPK; MDH; GPI; PGM	2.53,54,60,61
RW	GPI; EST-A	2.56, 62

(iii) Sterile Dark Group 7

Eight enzyme stains used on four buffer systems produced similar bands for the three isolates from this sterile group (Table 2.21, Figures 2.55-2.66)

Table 2.21 Isozyme systems which grouped isolates of SDG 7

Buffer System	Enzyme system	see Figure
AC	CPK; G6P; GPI; MDH;	2.53,54,60,63
RW	G6P; GPI; PGM	2.55,56,57
PH	AK; G6P; ICD	2.64,65
TC	G6P; GDH; ICD	2.58,63,65,66

(iv) Sterile Dark Group 8

Five enzyme stains used on two buffer systems produced similar bands for the three isolates from this sterile group (Table 2.22, Figures 2.54-2.63)

Table 2.22 Isozyme systems which grouped isolates of SDG 8

Buffer System	Enzyme system	see Figure
AC	CPK; G6P; MDH;	2.54,60,63
RW	GPI; PGM	2.56,57

(v) Sterile Hyaline Group 1

Three enzyme stains used on three buffer systems produced similar bands for the six isolates from this sterile group (Table 2.23, Figures 2.60-2.65)

Table 2.23 Isozyme systems which grouped isolates of SHG 1

Buffer System	Enzyme system	see Figure
AC	CPK	2.60
RW	G6P	2.63
TC	G6P; ICD	2.63,65

(vi) Sterile Hyaline Group 3

Five enzyme stains used on four buffer systems produced similar bans for the three isolates from this sterile group (Table 2.24, Figures 2.54, 2.65)

Table 2.24 Isozyme systems which grouped isolates of SHG 3

Buffer System	Enzyme system	see Figure
AC	G6P; MDH; PGM	2.54,61,63
RW	G6P; GPI	2.55,56
PH	G6P	2.58
TC	G6P; ICD	2.58, 63,65

Initial results seem to indicate that isolates grouped by morphology are also grouped together by isozyme analysis which is further evidence that these are valid taxonomic groupings.

After this initial screening experiment, further electrophoresis work using the best enzymes and buffer systems identified from this work should be undertaken for each sterile group with more isolates and replication to enable reproducible isozyme band patterns. These bands are then scored and coefficients of similarities (CS) values are calculated that measure the degree of similarity in terms of the average numbers of alleles in common within and between species or groups of isolates. Isolates of the same species and groups will have similar high CS values which allow isolates to be statistically separated into groups (Bonde *et al.* 1993). Unfortunately this could not be completed within the parameters of this study but any future work with these isolates could use isozyme analysis as another tool to enable groupings to be confirmed.

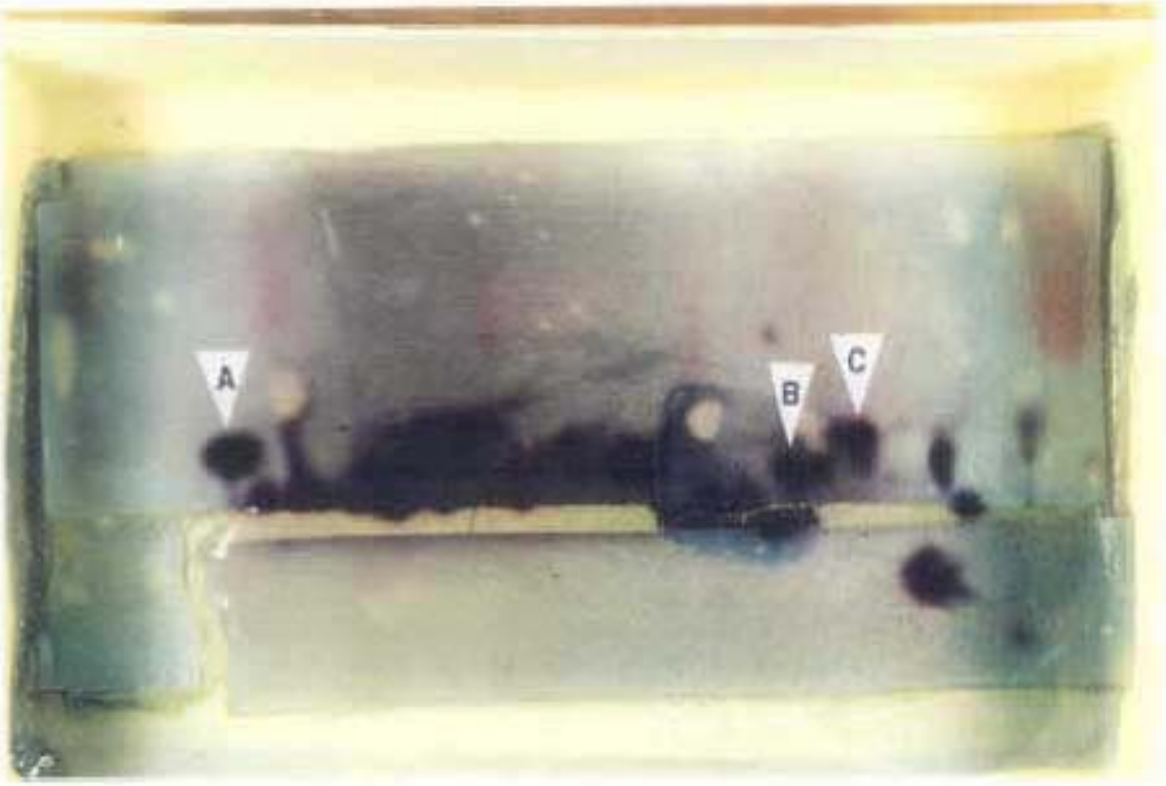


Figure 2.53 (A) SDG 3 (live mycelium extracts), (B) SDG 5, (C) SDG 7, (freeze dried mycelium samples) stained using GPI/ AC system.

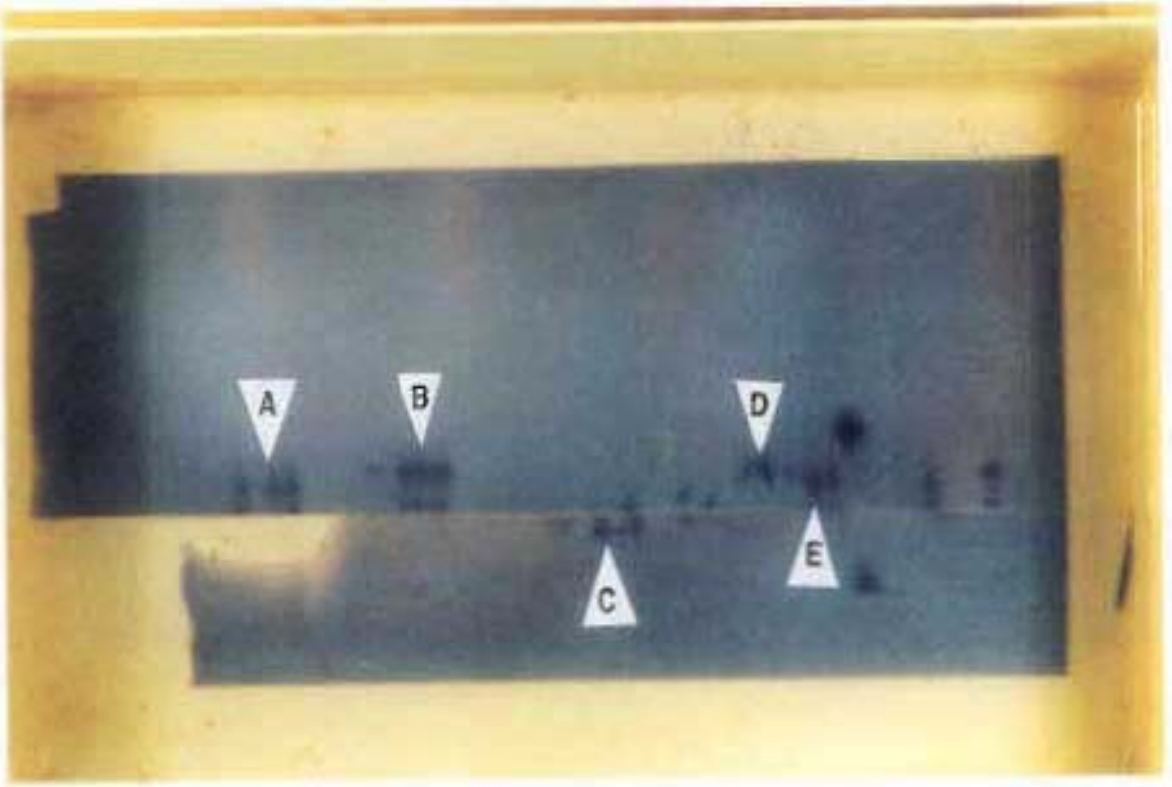


Figure 2.54 (A) SDG 8, (B) SDG 7 (live mycelium extracts), (C) SDG 3, (D) SHG 3, (E) SDG 5 (freeze dried mycelium samples) stained using MDH/ AC system.

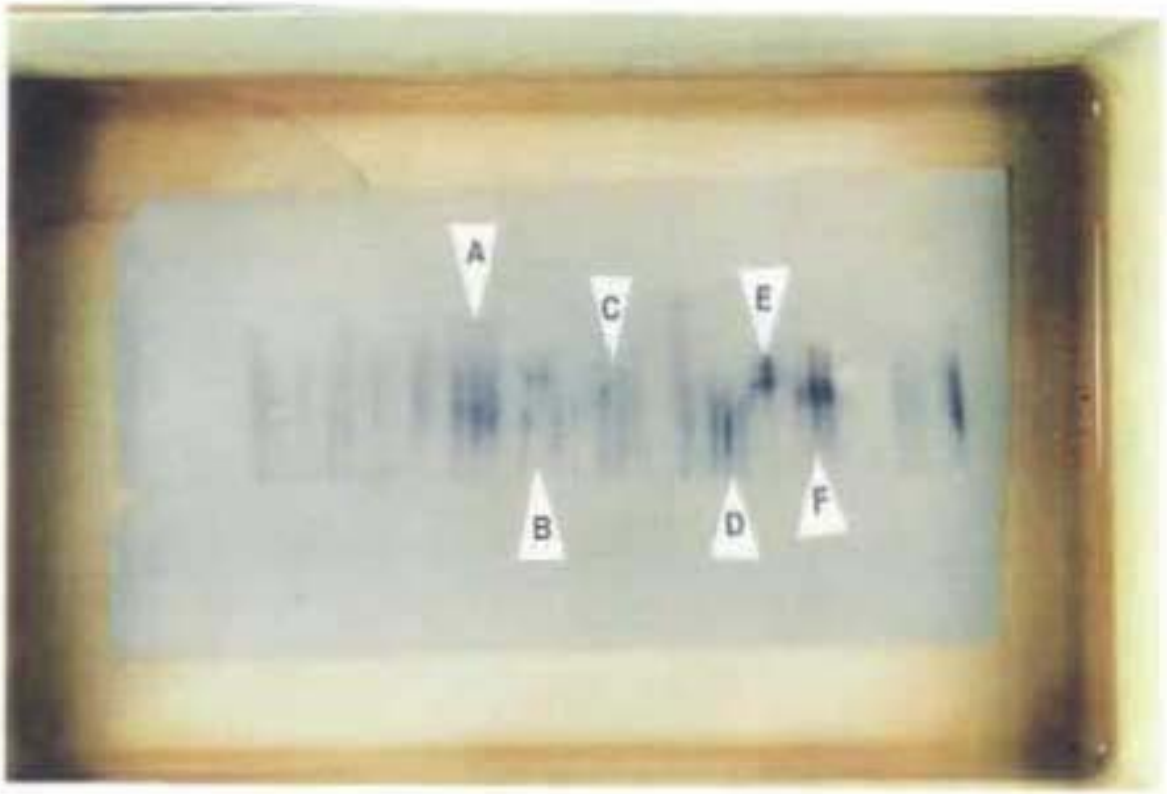


Figure 2.55 (A) SDG 7 (live mycelium extracts), (B) SHG 1, (C) SDG 3, (D) SHG 3 (E) SDG 5, (F) SDG 7 (freeze dried mycelium samples) stained using G6PDH/ RW system.

Figure 2.56 (A) SDG 3, (B) SDG 8, (C) SHG 3, (D) SDG 7 (live mycelium extracts), (E) SHG 1, (F) SDG 3, (G) SDG 8, (H) SDG 5, (I) SDG 7 (freeze dried mycelium samples) stained using GPI/ RW system.

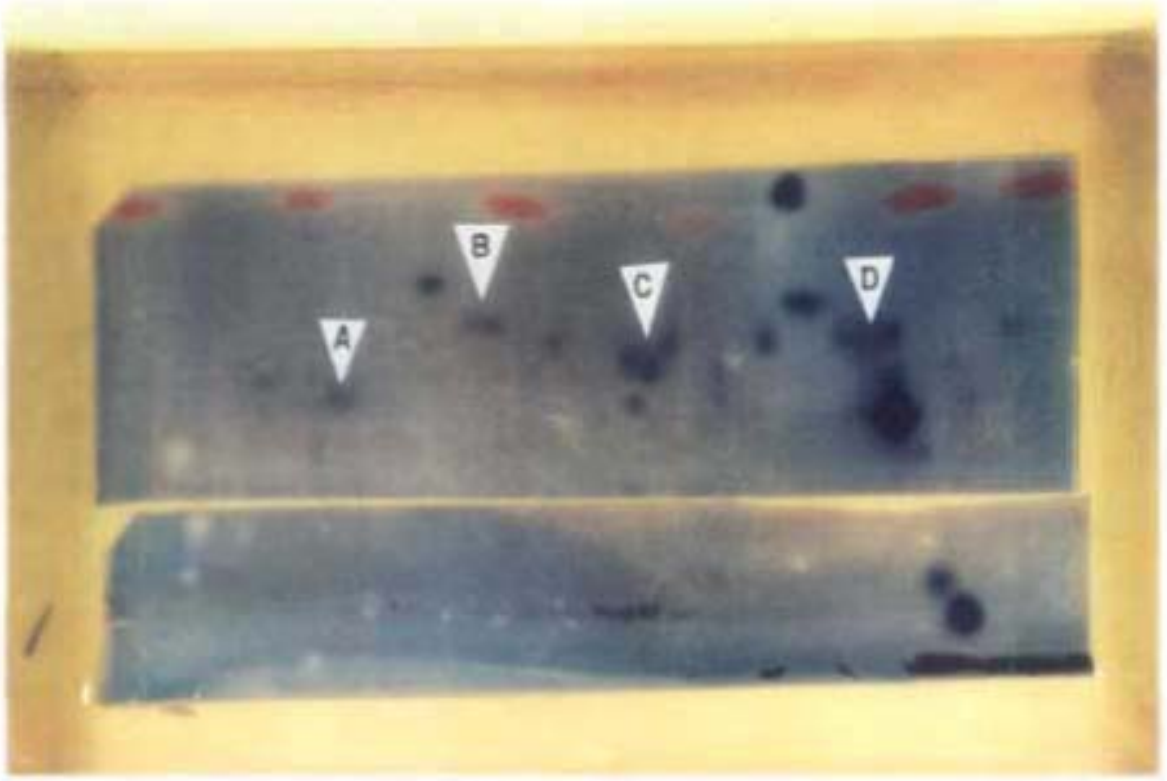


Figure 2.57 (A) SDG 8, (B) SDG 7 (live mycelium extracts), (C) SDG 3, SDG 7 (freeze dried mycelium samples) stained using PGM/ RW system.

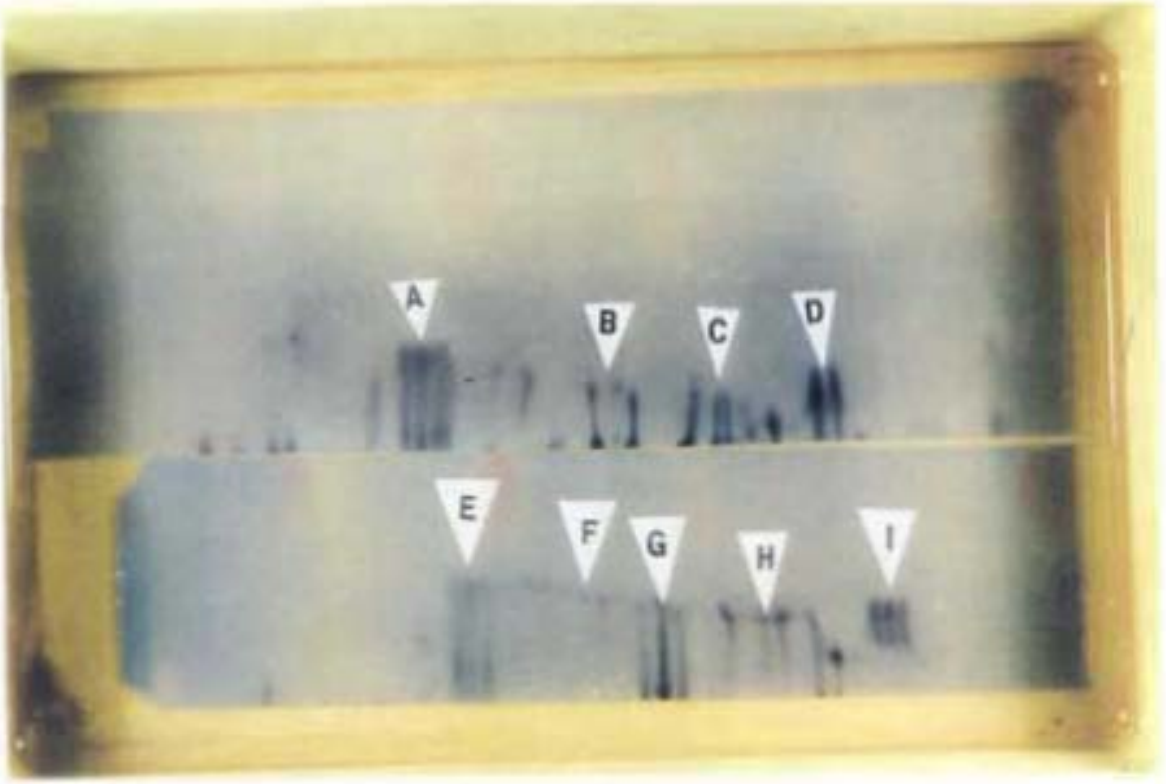


Figure 2.58 (A) SDG 7, (B) SDG 3 (live mycelium extracts), (C) SHG 3, (D) SDG 7 (freeze dried mycelium samples) stained using G6PDH/PH system, (E) SDG 7 (live mycelium extracts), (F) SHG 1, (G) SDG 3, (H) SHG 1 (I) SDG 7 stained using G6PDH/TC system.

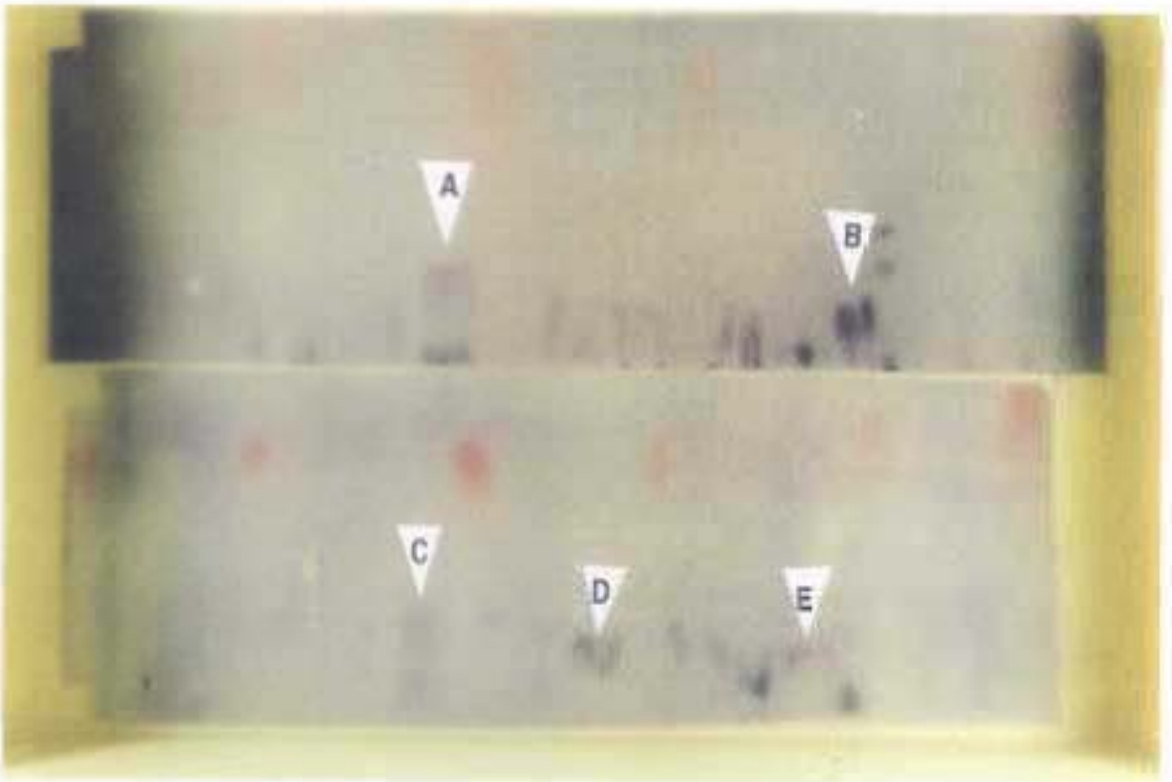


Figure 2.59 (A) SDG 7, (live mycelium extracts), (B) SHG 3, (freeze dried mycelium samples) stained using CPK/AC system, (C) SDG 7 (live mycelium extracts), (F) SDG 3, (G) SDG 7, stained using AC/PH systems.

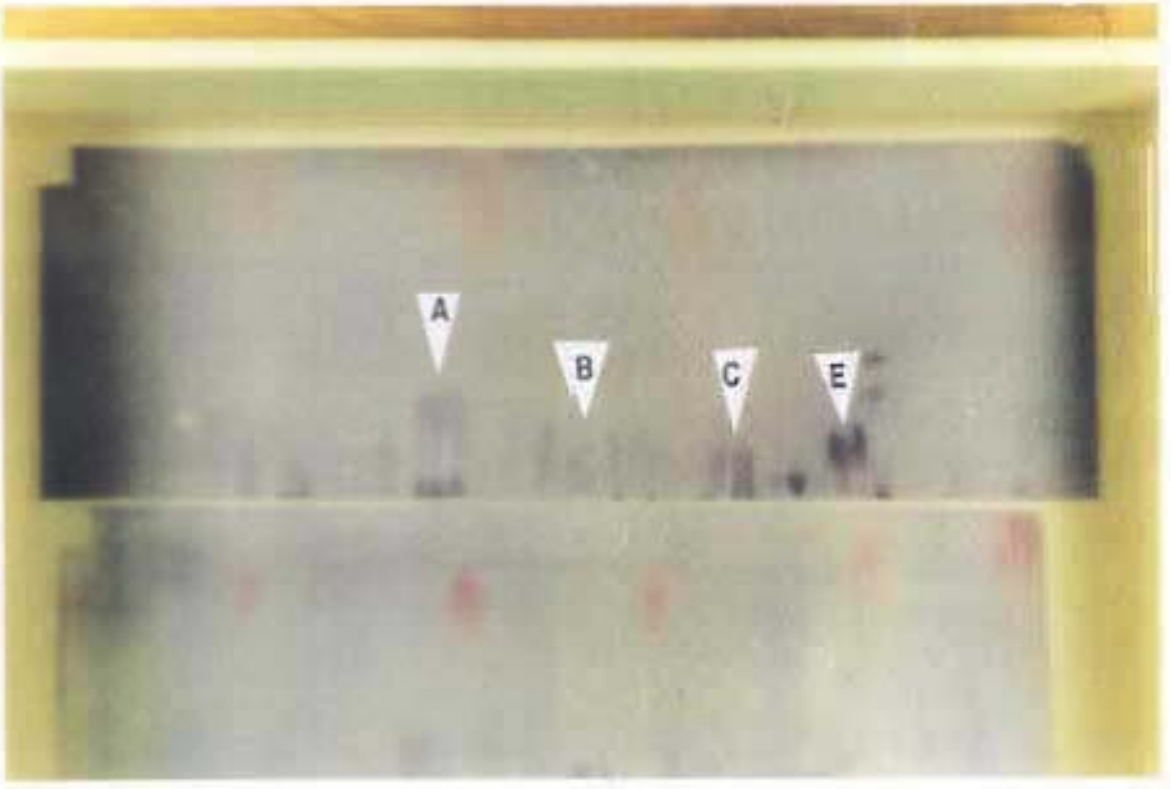


Figure 2.60 (A) SDG 7, (live mycelium extracts), (B) SHG 1, (C) SDG 8 (D), SDG 7 (freeze dried mycelium samples) stained using CPK/AC system.

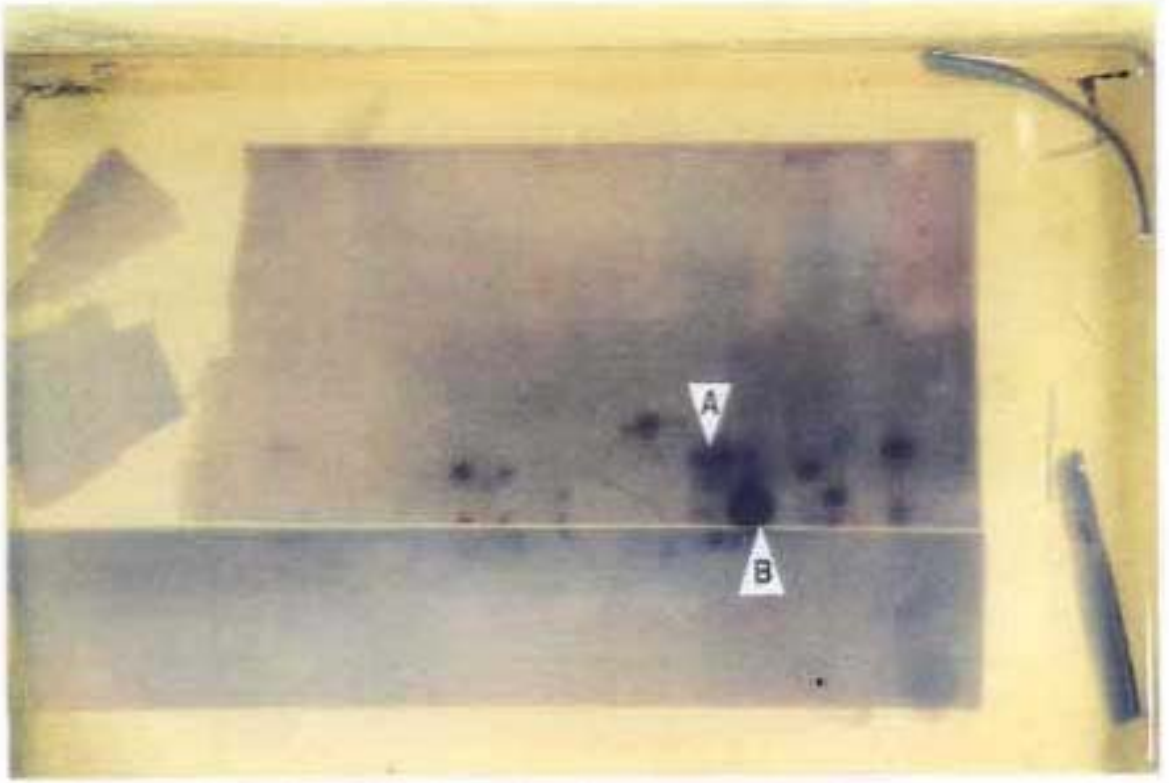


Figure 2.61 (A) SHG 3, (B) SDG 5, (freeze dried mycelium samples) stained using PGM/AC system.

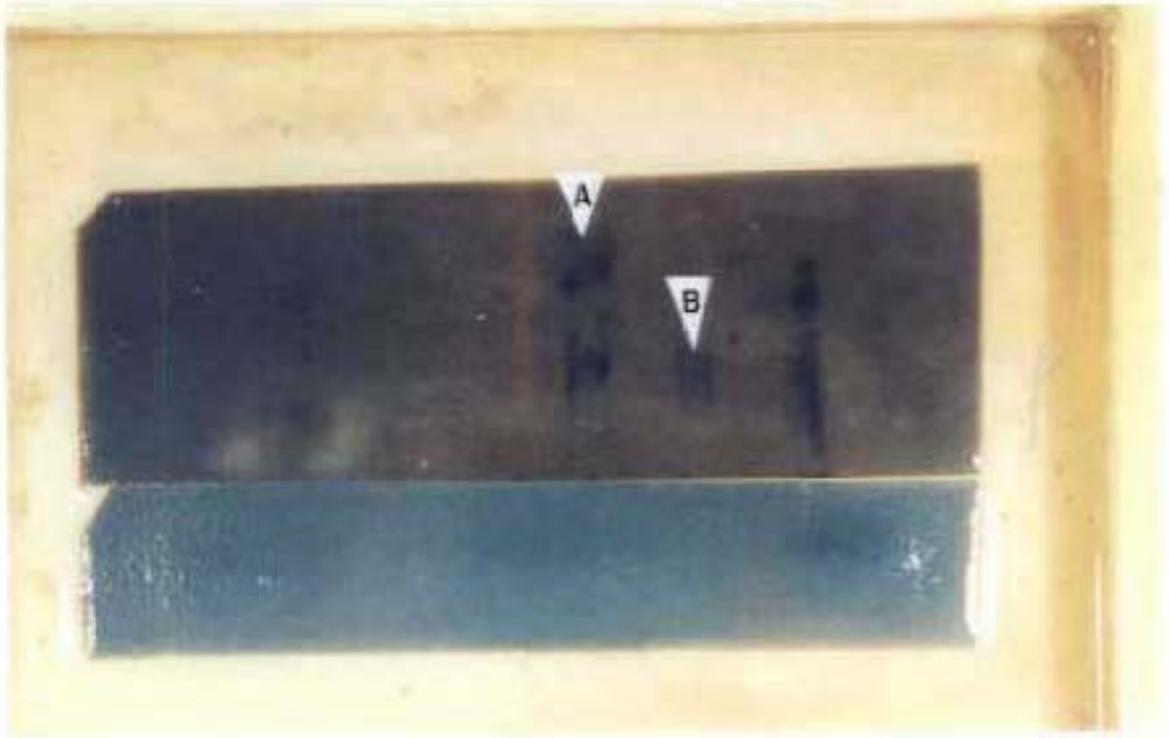


Figure 2.62 (A) SDG 3, (B) SDG 5 (freeze dried mycelium extracts) stained using EST-A / RW system.

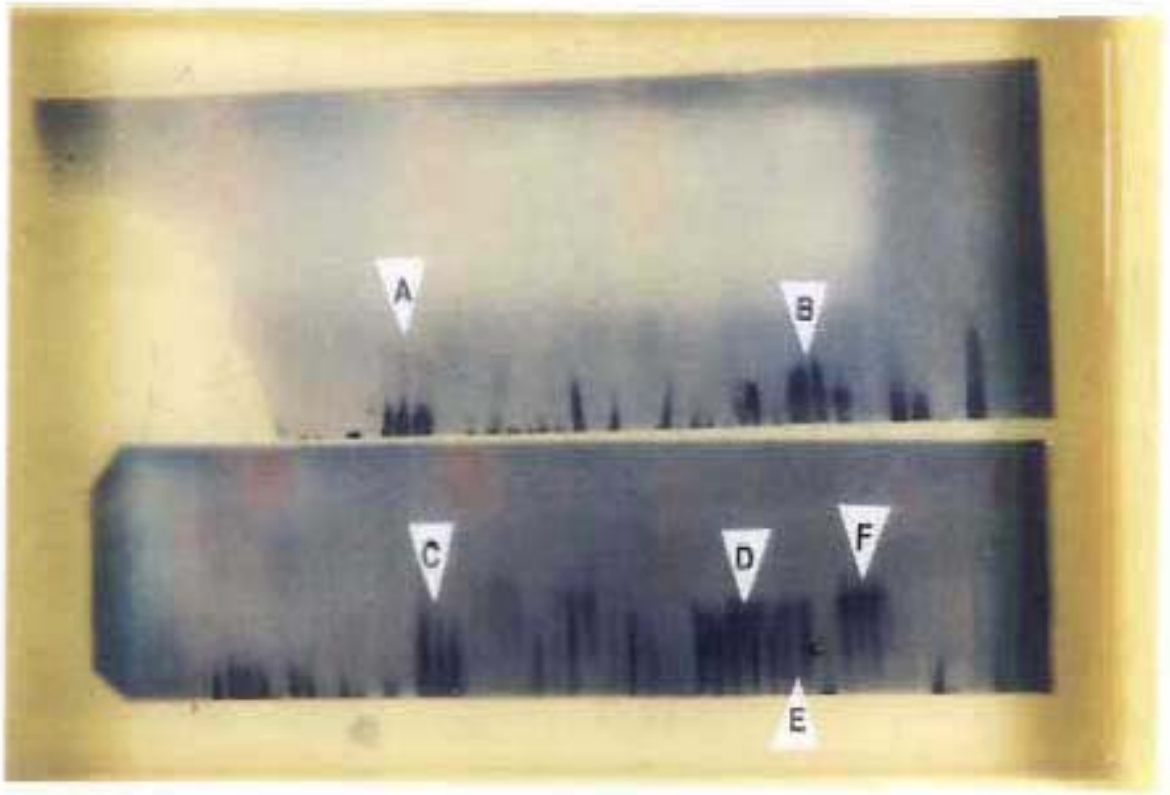


Figure 2.63 (A) SDG 7, (live mycelium extracts), (B) SHG 3, (C) SDG 7 (freeze dried mycelium samples) stained using G6PDH/TC system, (D) SDG 7 (live mycelium extracts), (E) SDG 8, (F) SHG 3, (G) SDG 7 stained using G6PDH/AC system.

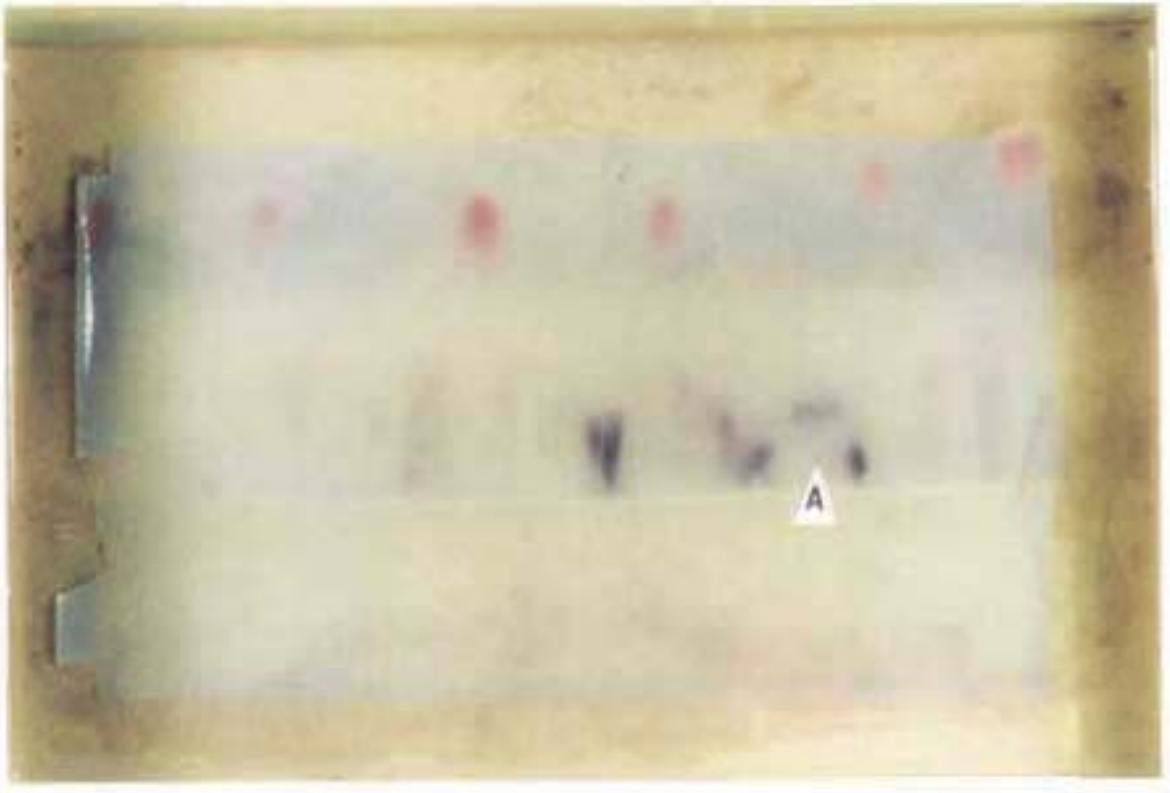


Figure 2.64 (A) SDG 7 (freeze dried mycelium extracts) stained using AK / PH system.

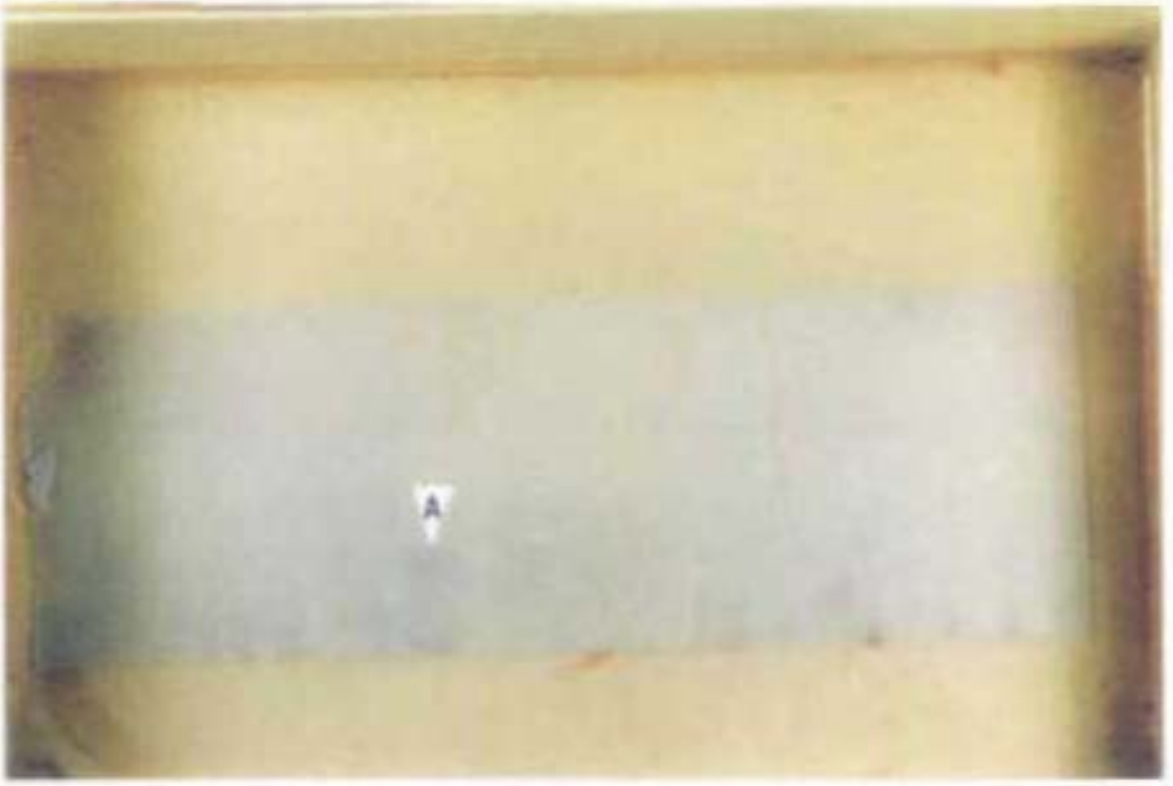


Figure 2.65 (A) SDG 7 (live mycelium extracts) stained using AK / PH system.

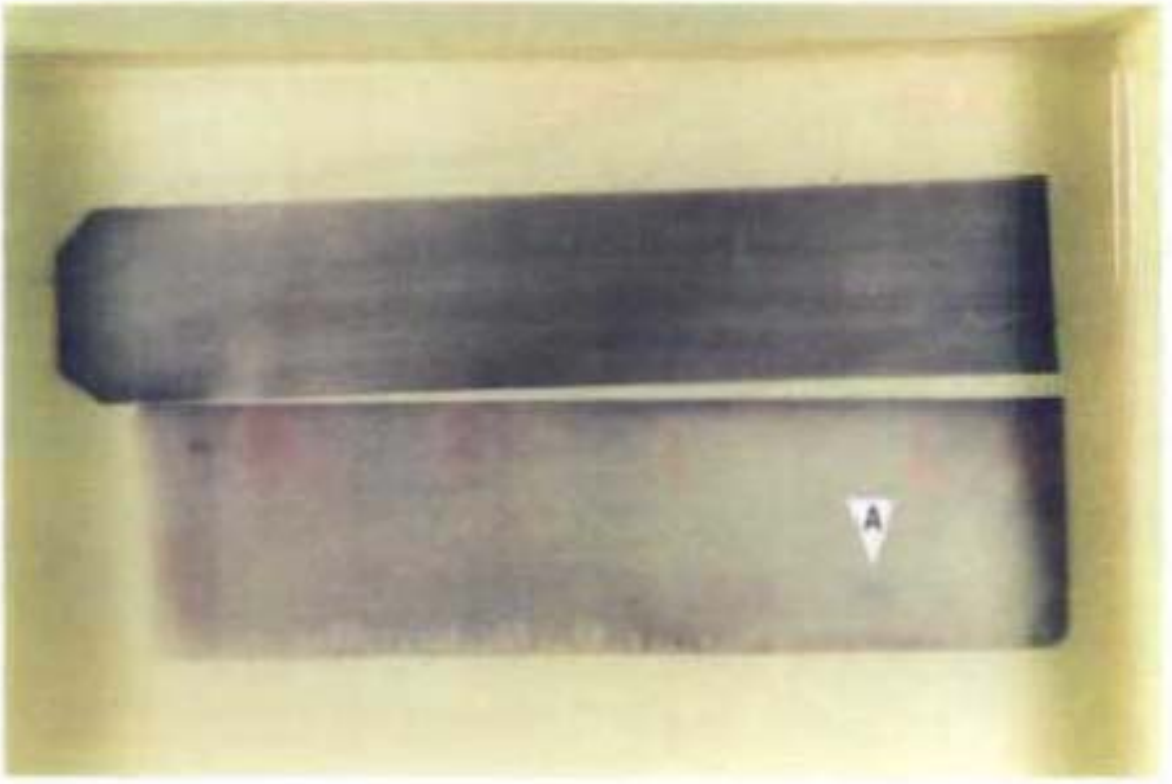


Figure 2.66 (A) SDG 7 (freeze dried mycelium extracts) stained using GDH / TC system.

2.3.4 TAXONOMY OF TWO FUNGI INDUCED TO SPORULATE AFTER BEING ISOLATED FROM ROOTS AS STERILE MYCELIUM.

2.3.4 (a) *Thozetella tocklaiensis* (SDG 10)

The following results have been reported (Waipara *et al.* 1996d, Appendix 9). Size of sporodochia of *T. tocklaiensis* varied from 500 µm to 2000 µm in diameter and up to 1000 µm in height (Figure 2.67). Sporodochia were produced 3-4 weeks after inoculation and were partly immersed in the agar (Figure 2.68). Elongated dark hyphal cells were tightly packed to form a stroma which produced conidiophores bearing a globose slimy white mass of conidia and microawns (Figure 2.69). Sporodochia initially appeared slimy and white which became greenish grey or glabrous (27-28 A-B3) as the cultures aged. Conidia were smooth, hyaline, guttulate, one celled, sigmoid to falcate in shape, and possessing a single filiform setula at each end (Figures 2.70-2.72). They ranged in size from 13-20 x 2-3.5 µm, the setula measuring 4-10 µm. Conidia were produced singly from a simple single opening at the apex (Figures 2.73 - 2.75), Conidiogenous cells were 8-19 µm x 1.5-4.0 µm, slightly tapered to a bluntly rounded apex (Figures 2.73, 2.74). Conidiophores were closely compacted and often partly fused with parallel branching to give a palisade appearance within the sporodochium (Figure 2.74). Hyphae associated with conidiophores also produced hyaline, irregular L-shaped sterile microawns 22-60 x 2.5-5.5 µm, the basal end of which was tapered and rugose curving to a smooth, blunt apex (Figure 2.70). Electron microscopy clearly showed the origin of these microawns, which had previously been speculative (Pirozynski and Hodges 1973). Microawns were produced prior to sporulation, and only by the outer or excipular hyphae of the sporodochium, while inner conidiophores produced only conidia (Figure 2.72). Microawns were originally described as sterile awn-shaped setae (Agnihothrudu 1958), and the term microawn was subsequently proposed by Pirozynski and Hodges (1973), to indicate their gross morphological similarity to awns of certain Gramineae. It was suggested the function of these sterile microawns was to aid dispersal of conidia, or act as deterrents to spore-feeding animals (Pirozynski and Hodges 1973). As they are produced before sporulation, and on the periphery of the sporodochium, it would appear that a protective function is more likely. The size range of all diagnostic structures was the same on all media, however the average varied (Table 2.25). Chlamydospores were absent on all media and did not appear even in 4-month old cultures. This contrasts with Agnihothrudu (1958) who described abundant production of terminal and intercalary oval chlamydospores on all media. As chlamydospore production was not mentioned in the descriptions of *Thozetella* species by Pirozynski and Hodges (1973), it is possible that the absence of chlamydospore production was peculiar to strains isolated from pasture roots.

Table 2.25 Average dimensions of conidia, setae, conidiogenous cells, microawns of *T. tocklaiensis* on different media. Measurements based on means of 100 observations.

Media	Conidia (µm)	Setae (µm)	Conidiogenous cells (µm)	Microawns (µm)
OA	16.4 x 2.7	6.2	16.8 x 2.3	36.5 x 2.8
PCA	15.0 x 2.9	6.0	13.3 x 1.8	37.1 x 3.5
HA	14.7 x 2.8	6.3	16.3 x 2.0	44.2 x 3.0
SP	16.5 x 3.0	7.4	17.5 x 2.9	40.1 x 3.6
Range	**13-17(-20) x 2.0-3.5**	**4-10**	**8-19.5 x 1.5-4.0**	**22-60 x 2.5-5.5**

Previous reports have suggested that *T. tocklaiensis* is saprophytic in nature and found in plant litter and soil. However, the intra- and intercellular invasion of root cells by hyphae seen in the present study indicate that it is capable of infection. Many root-invading fungi are very weak pathogens or saprophytes which aid decomposition of senescing plant roots (Waid 1974) and as such are beneficial in stimulating root turnover.

Isolates of *T. tocklaiensis* obtained from pasture grasses in this study differ from previous descriptions (Agnihothrudu 1958, Pirozynski and Hodges 1973) in that they did not sporulate easily and did not produce chlamydospores. Further morphological examination of these isolates may be necessary to determine if they are a sub species which does not produce chlamydospores. However, the morphological characters clearly identify this fungus as *Thozetella tocklaiensis* and the dimensions of conidia and associated structures are in the same range as the strains originally described.

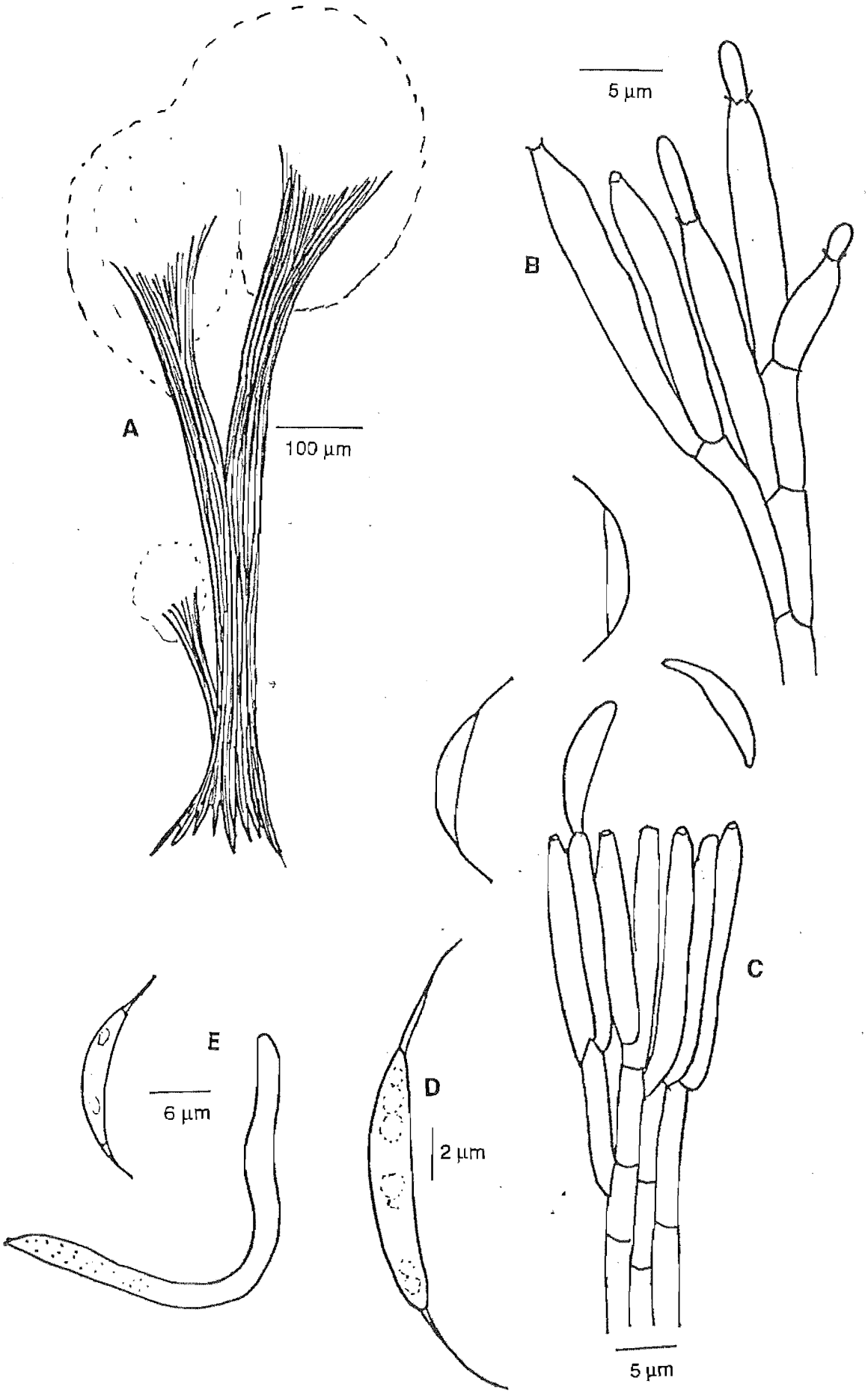


Figure 2.67 *Thozetella tocklaiensis,* (A) Sporodochium, (B) conidiogenous cells, (C) conidiophore, (D) conidium with terminal setae, (E) sterile microawn and conidium.

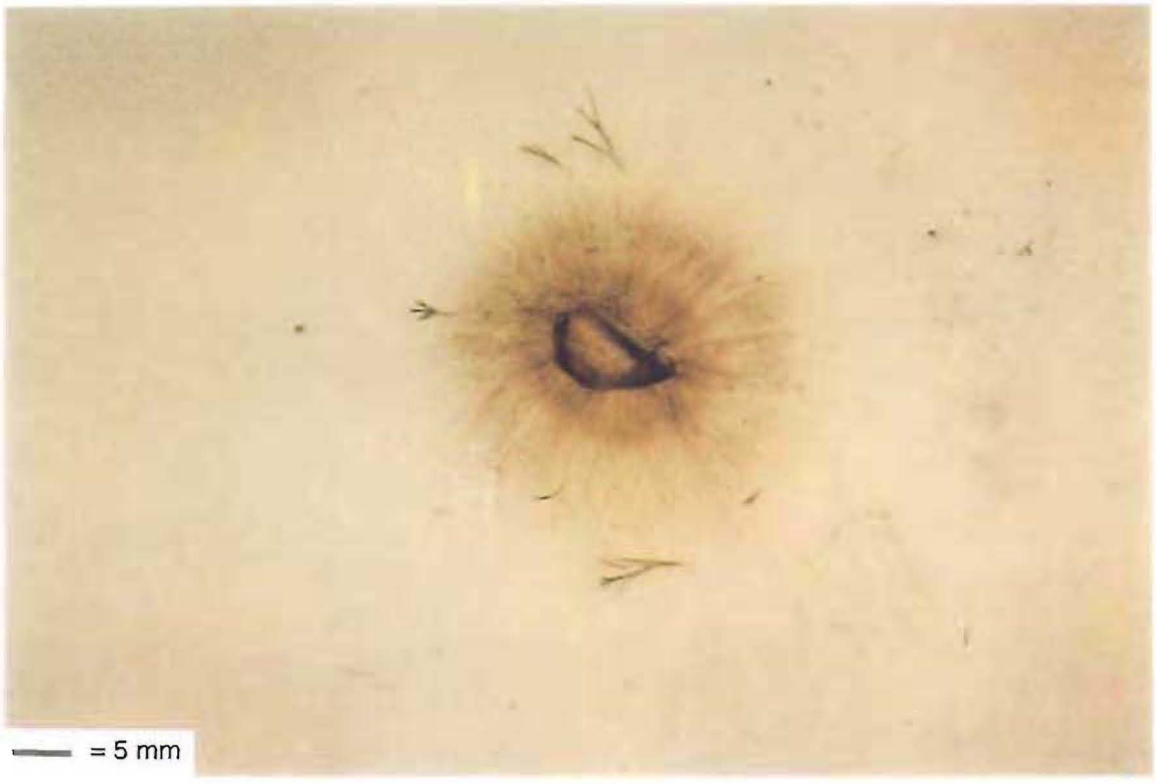


Figure 2.68 Immature sporodochium of *T. tocklaiensis* immersed in potato carrot agar.

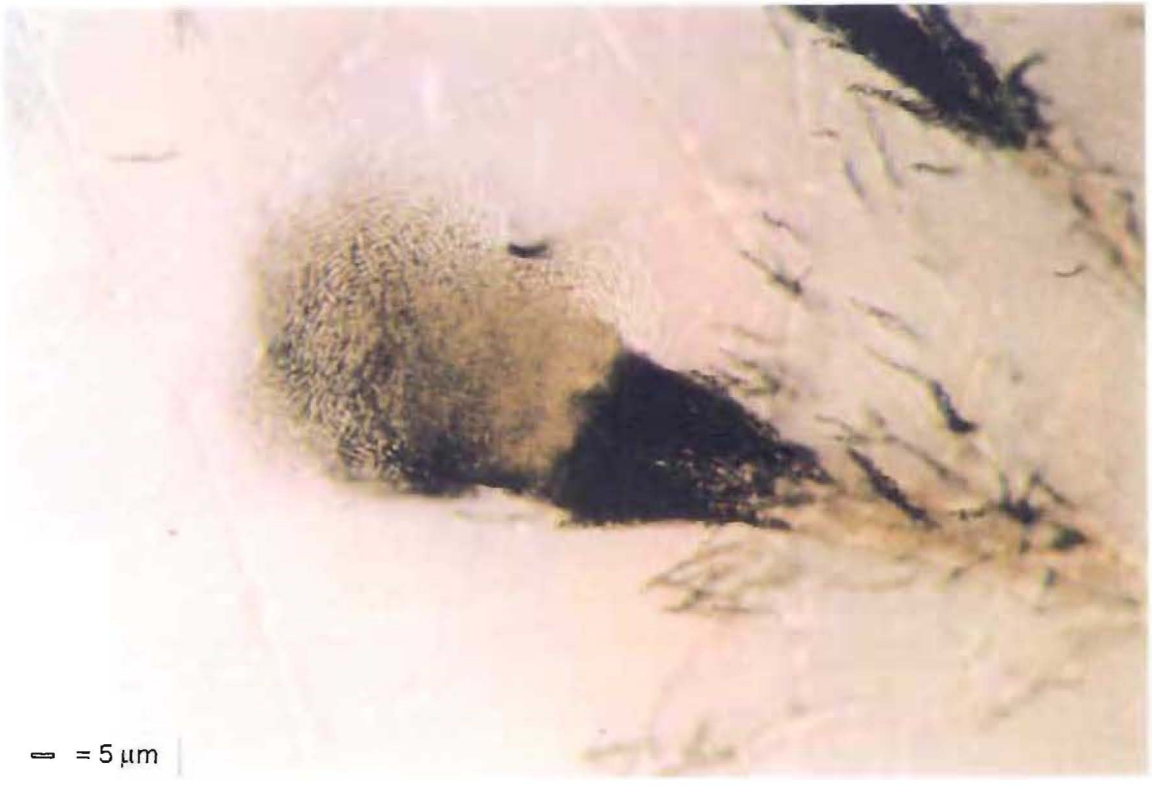


Figure 2.69 A mass of conidia produced by *T. tocklaiensis* in tightly packed conidiophores.

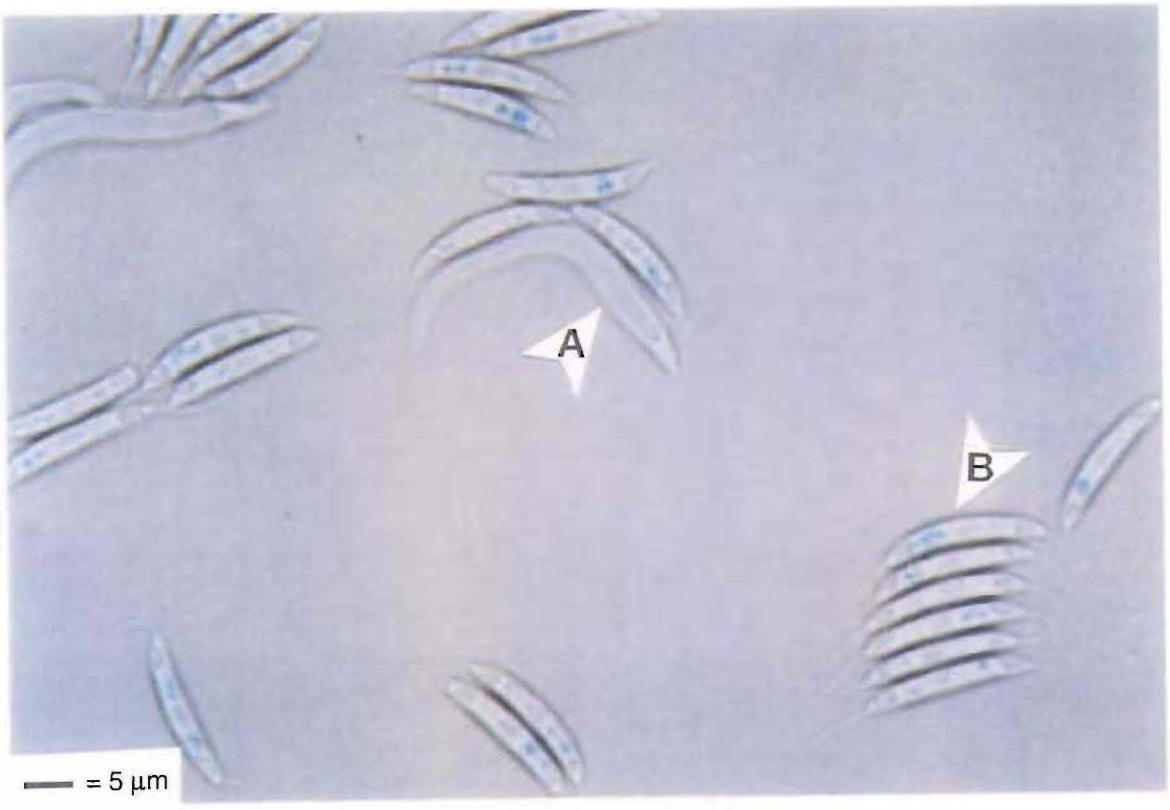


Figure 2.70 ***Thozetella tocklaiensis*** **(A), L-shaped sterile microawn; (B), Conidia.**

Figure 2.71 Electron micrograph of a mass of conidia produced by tightly packed conidiophores of ***T. tocklaiensis***.

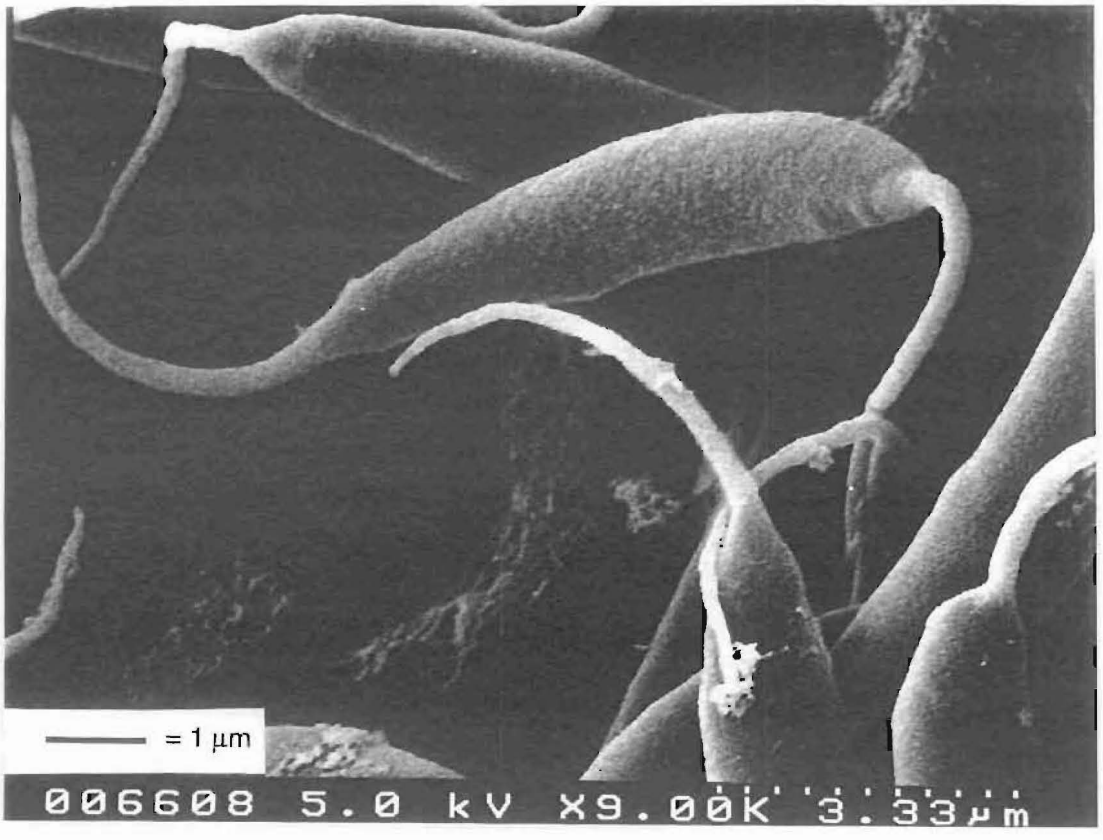


Figure 2.72 Electron micrograph of *T. tocklaiensis* of conidia with terminal setula.

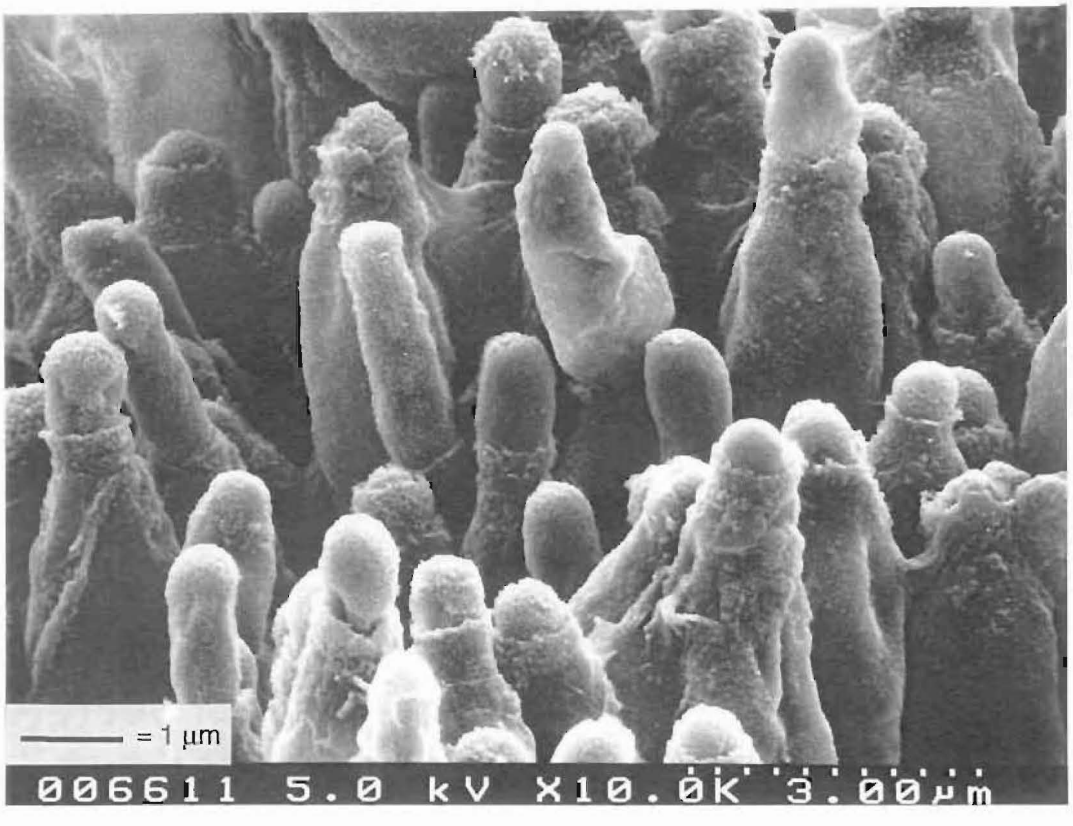


Figure 2.73 *Thozetella tocklaiensis*, conidiogenous cells with immature conidia being produced at the apex

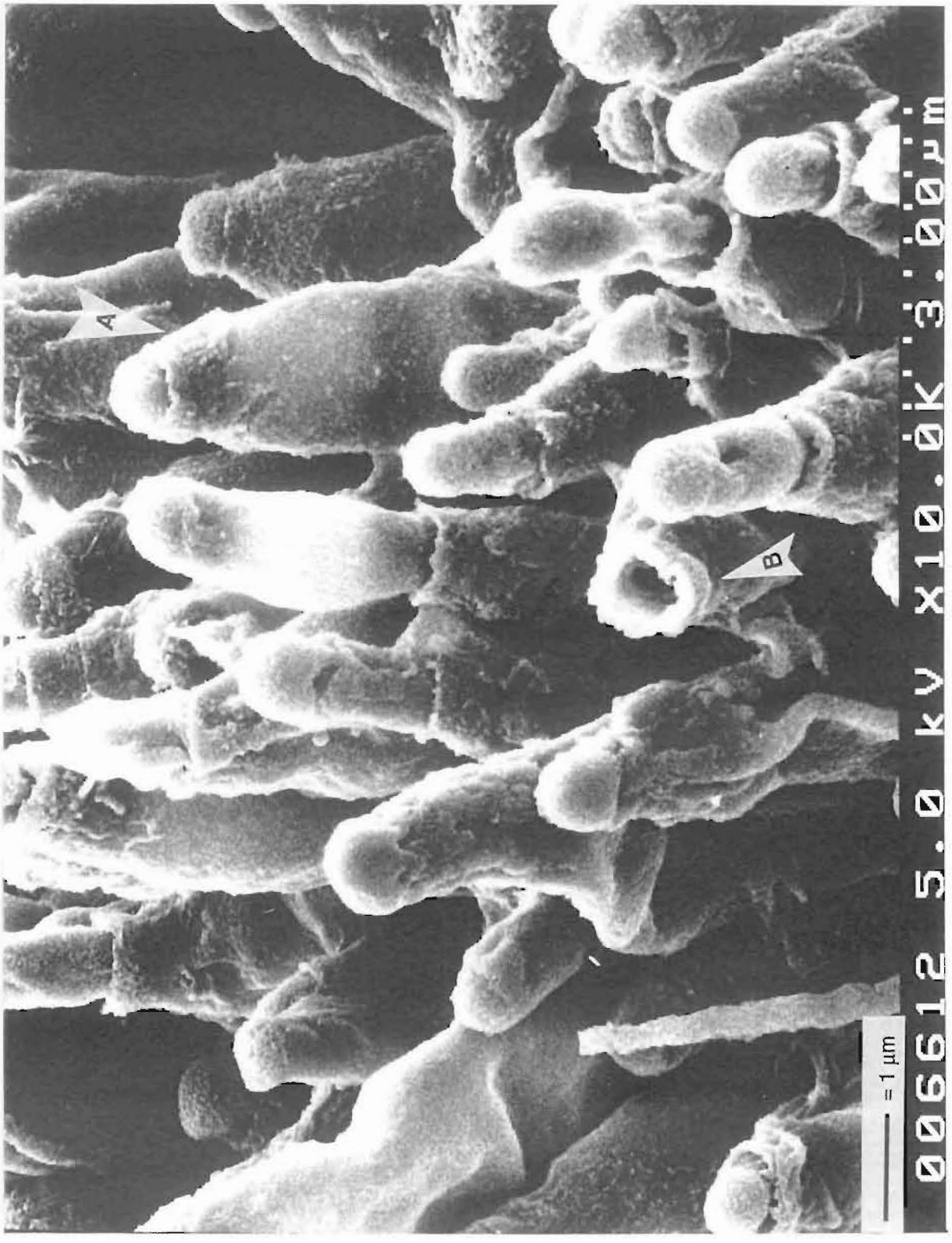


Figure 2.74 Sporodochia of *T. tocklaiensis*, (A), conidiogenous cells producing conidia, surrounded by (B), a palisade of sterile microawns.

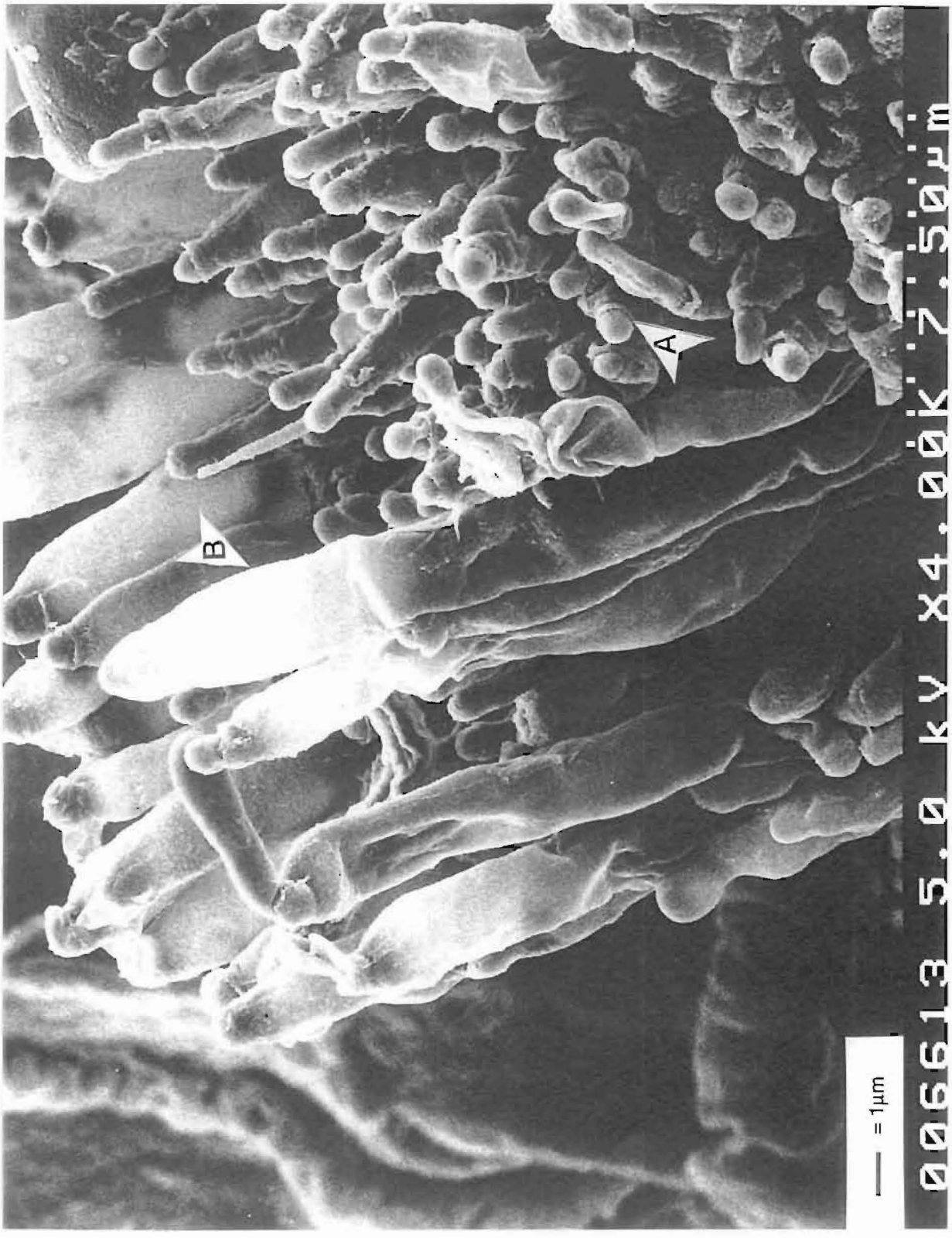


Figure 2.75 Sporodochia of *T. tocklaiensis*, (A), conidiogenous cells producing conidia, (B) apical opening of conidiogenous cell.

2.2.4 (b) *Phialophora* sp.

Phialophora Medlar is a hyphomycete genus characterised by the presence of a prominent cup-like collarette at the apical mouth of the conidiogenous cell (or phialide). The collarette is part of the conidiogenous cell wall and its size is dependent upon the quantity of outer wall tissue left after the formation of the initial conidium (Cain 1952, Schol-Schwartz 1970, Cole and Kendrick 1973).

Both colonies on PCA slopes were pale orange (6 A 3-7, Figure 2.17) with patches of white powdered growth within the colony where production of conidiophores and conidia had occurred. Aerial mycelium of PCA colonies sparse and immersed mycelium composed of hyaline frequently branched hyphae and smooth-walled. Chlamydospores were absent but monilioid cells were often produced (Figure 2.76). The branching pattern of hyhae with conidiophores was irregular and dendritic (Figures 2.76-2.77). Conidiophores were usually branched (polyphialidic), with septate cells of varying size, often slightly swollen, septa occurred at varying intervals (2.0-8.5µm). Conidiophores were smooth-walled and measured 4.0 -13 x 1.0 - 2.4 µm (Figures 2.78, 2.79). Conidiogenous cells were borne in irregular clusters (up to 13) and were subcylindrical or sausage-shaped (Figures 2.79, 2.80). The basal septum of each cell was slightly constricted (maximum width of 3.4 µm), with a roundly tapered to a sharply waisted apex at the conidiogenous locus where each conidiogenous cell terminated with a tubular, parallel-sided, hyaline collarette (Figures 2.79, 2.80). Conidiogenous cell walls were hyaline, smooth walled measuring 1.8-3.4 x 0.5 - 1.8 µm and the collarettes measuring 1.0-2.2 µm (Figure 2.80). Conidia were produced in dry heads (Figure 2.81) and two kinds of 0-septate conidia were observed. The first conidium developed within the unbroken extension of the conidiogenous cell, was hyaline, smooth-walled and obovoid in shape and measured 1.2-2.4 µm x 0.5-1.5 (Figure 2.82). After the apex of the collarette had ruptured this initial conidium (type I) was released from each coniodogenous cell and all subsequent conidia (type II) were variable in size (0.5-2.2 µm x 0.4-0.7 µm), and shape ranged from spherical to cylindrical (Figure 2.83). The average dimensions of each of these structures are presented in Table 2.26.

Table 2.26 Dimensions (mean ± Standard deviation) of Condiophores, conidiogenous cell, collarette and conidia of *Phialophora* sp. isolates.

	Length µm	Width µm
Conidiogenous cell	2.6 ± 0.6	1.3 ± 0.3
Collarette	1.7 ± 0.4	-
Conidia Type I	2.1 ± 0.4	0.9 ± 0.4
Conidia Type II	1.7 ± 0.6	0.5 ± 0.1

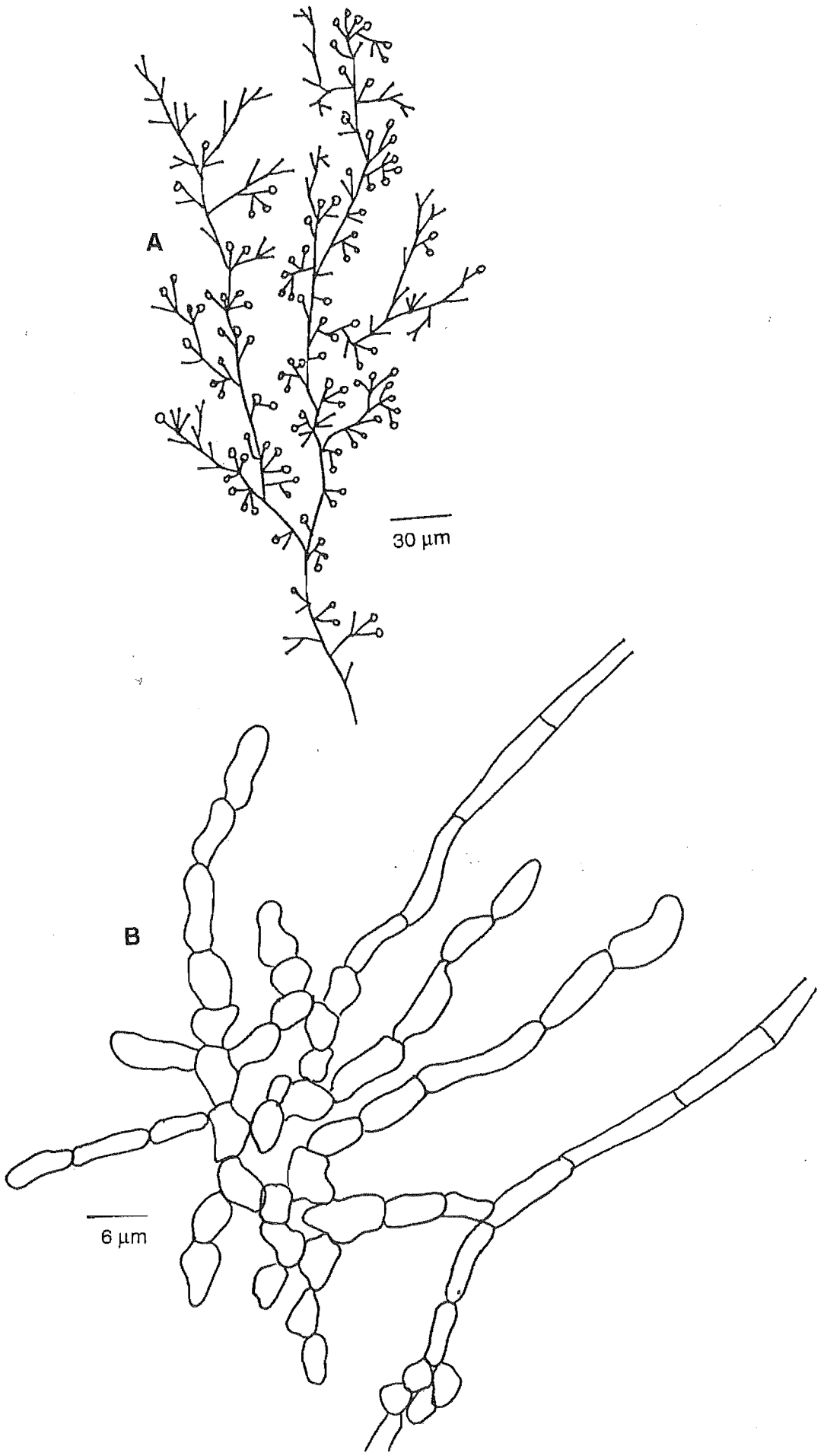


Figure 2.76 *Phialophora* **sp (A) Pattern of hyphal branching, (B) monilioid hyphae**

Figure 2.77 ***Phialophora*** **sp. irregular pattern of conidiophores borne on hyphae (20x).**

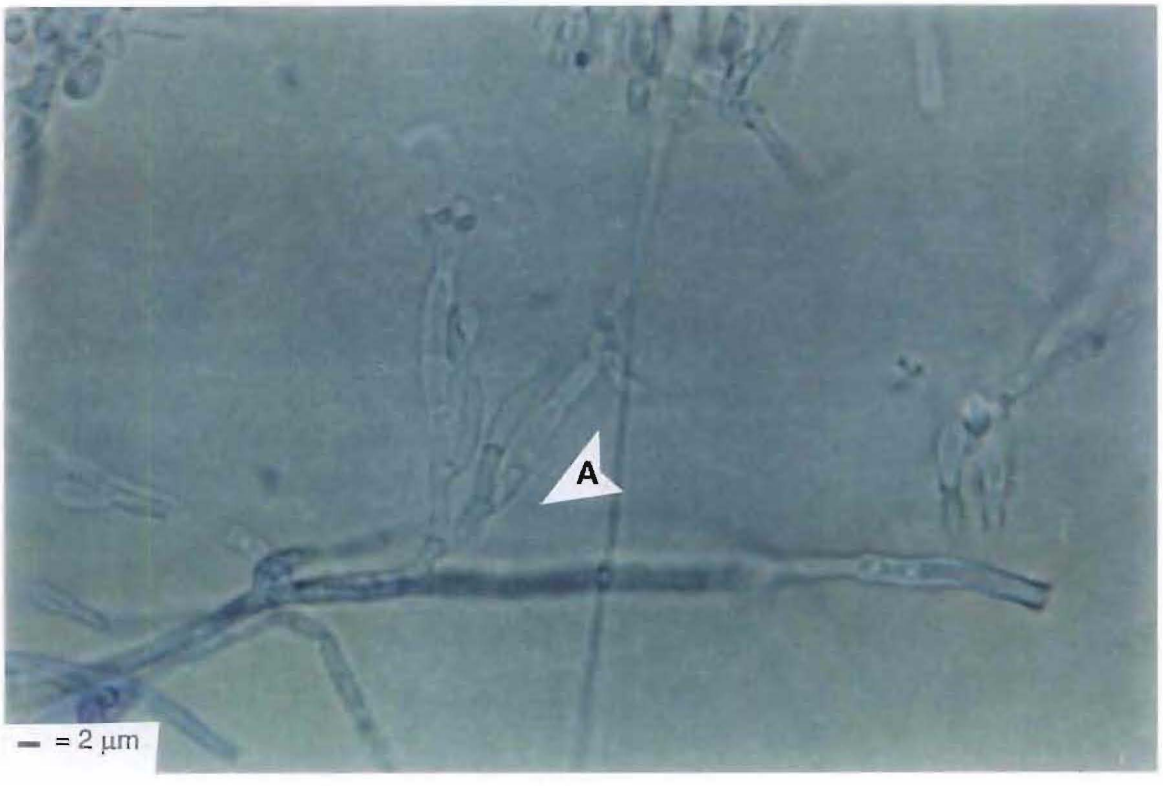


Figure 2.78 ***Phialophora*** **sp. (A) irregular branched conidiophores.**

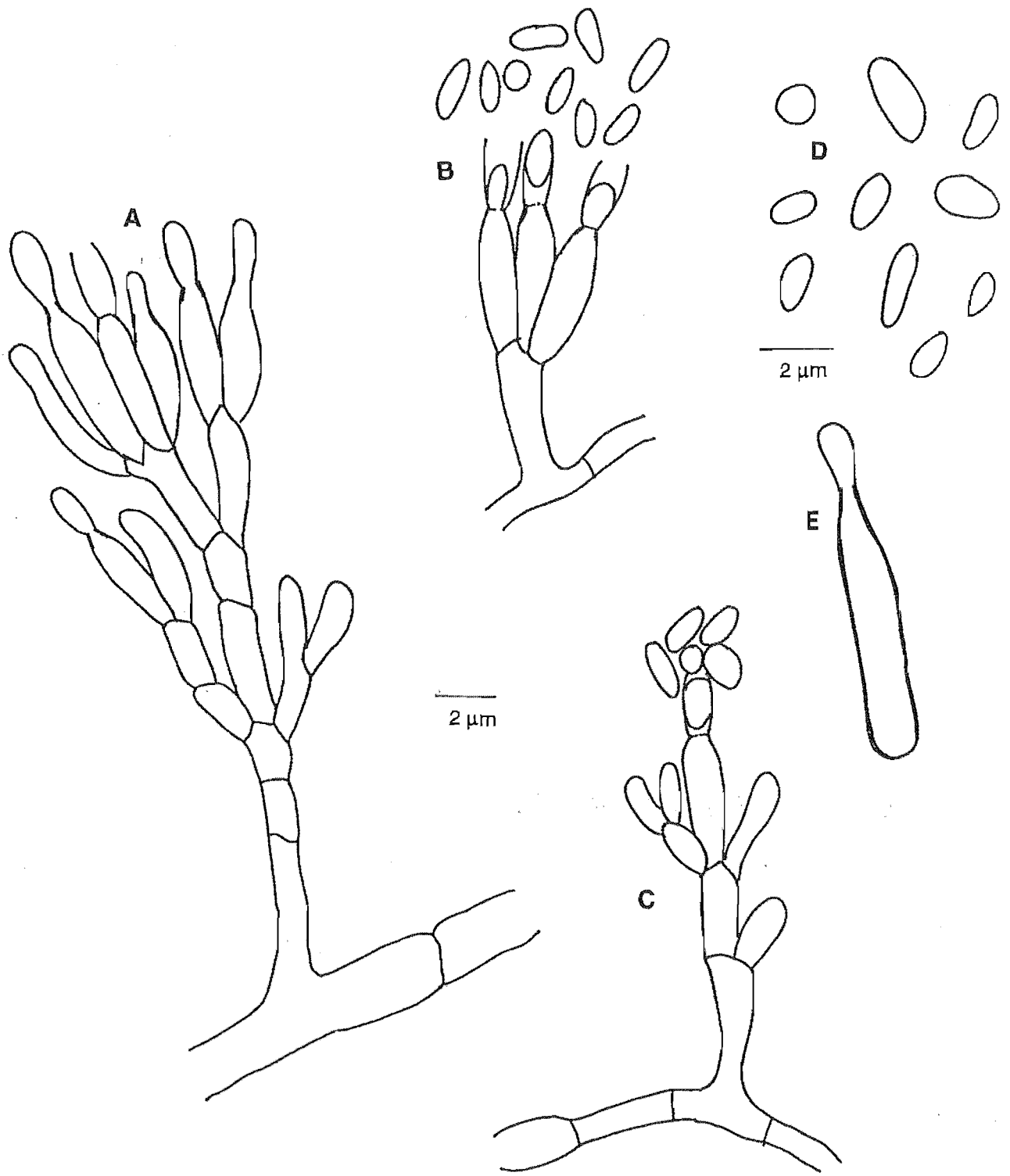


Figure 2.79 ***Phialophora*** **sp. (A-C) Conidiophores, (B) cylindrical conidiogenous cells terminated with cup-like collarettes, (D) conidia type 2, (E) conidiogenous cell producing an initial type 1 conidium.**

Figure 2.80 ***Phialophora*** **sp. Conidiophore (1000x), (A) parallel sided collarette.**

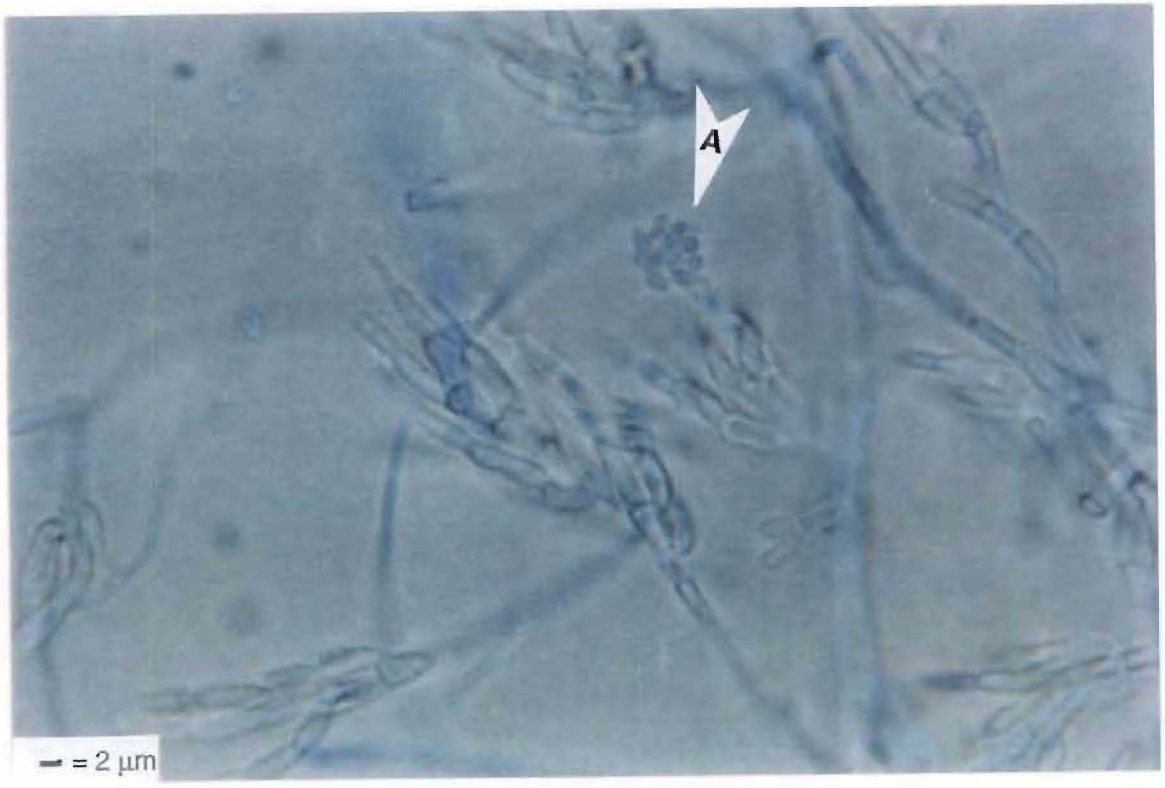


Figure 2.81 ***Phialophora*** **sp. Conidiophore (1000x), and (A) phialidic conidial production with conidia produced in dry heads.**

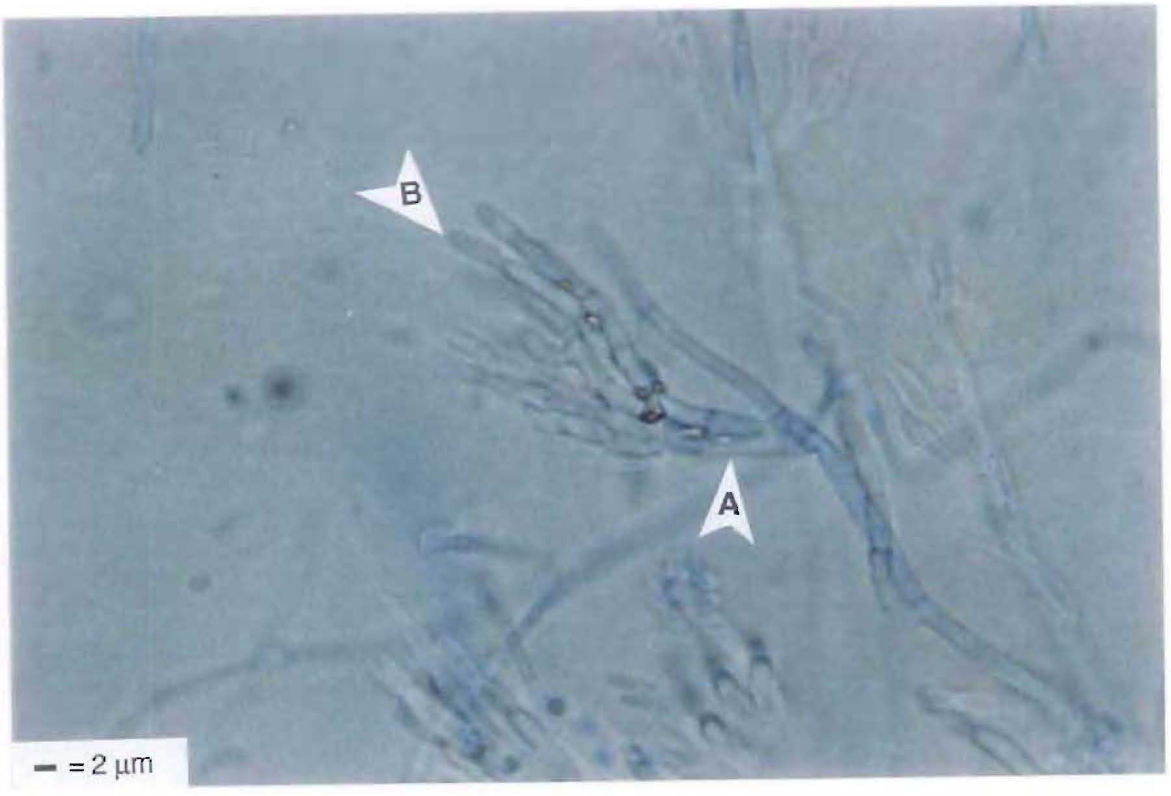


Figure 2.82 ***Phialophora*** **sp. (1000x) (A) cylindrical conidiogenous cell producing an initial conidium, (B) production of subsequent conidium (type 2).**

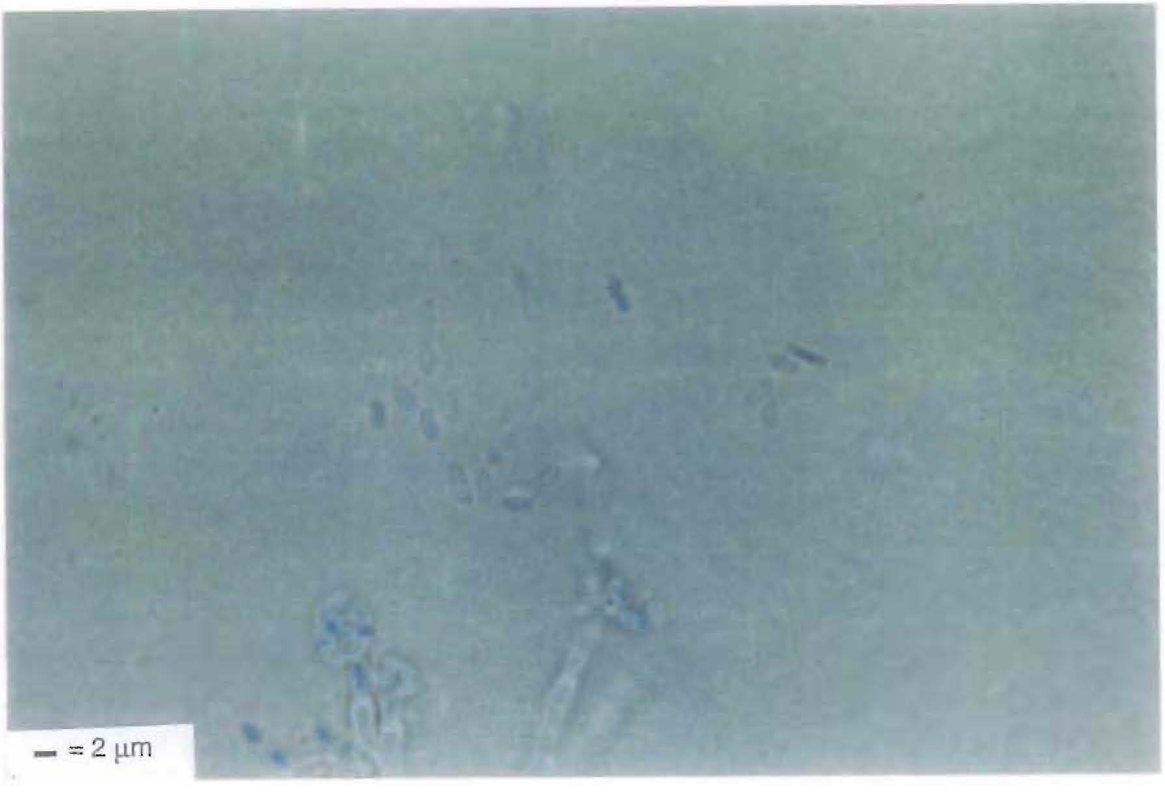


Figure 2.83 ***Phialophora*** **sp., one celled conidia of variable sizes and shapes (1000x)**

2.4 DISCUSSION

None of the sterile groups obtained in this study fit descriptions of existing genera of *Mycelia sterilia* or Agonomycetales. Morphological examination revealed distinct groups of similar isolates could be recognised and demonstrated these root-colonising sterile fungi to be a collection of taxonomic groups which may be equivalent to species or genera. The effect of fungicide on growth, carbon and nitrogen assimilation and isozyme electrophoresis also supported the morphological separation of isolates into sterile groups, but there was a significant proportion of sterile isolates which could not be grouped on the basis of cultural morphology.

The inherent difficulty in identifying asporogenous fungi in culture was clearly demonstrated with both *Thozetella tocklaiensis* and *Phialophora* sp., where initial mycelium was sterile but specific incubation requirements (medium type/ temperature shock plus UV light required for *T. tocklaiensis,* and long term incubation in culture for *Phialophora*) were needed to induce sporulation. The bulk of sterile isolates were not induced to sporulate in culture during this study and were therefore considered to be truly sterile isolates. Many studies lump sterile isolates into an amorphous collection of unrelated individuals because of the difficulty of identification (Parmeter 1965, Hall 1986), but effort should at least be made to distinguish truly sterile isolates from other species.

Molecular biology and genomic-based analyses that rely on comparisons of informative sequences of RNA and DNA could overcome the nonsporulation barriers to identification, in part, by distinguishing among or grouping together isolates with ambiguous morphological features or by establishing the genomic equivalency of isolates (Bills 1995). These molecular techniques may also overcome the reliance on isolation methods to evaluate fungal biodiversity which has been limited by nonsporulation of fungi which in turn is an impediment to identification (Bills 1995). Future work with DNA molecular techniques could be useful, particularly with sterile hyaline group 3, where isolates could be compared with *Oideodendron* species, using restriction fragment analysis of an amplified portion of ribosomal DNA (RAPDS analysis) to help identify the group. Isolates grouped as SHG 3 produced beaded hyphal structures similar to some species of *Oidiodendron* (Domsch *et al.* 1980). *Oidiodendron* is reported to be frequently isolated from soils and is often associated with plant roots (Domsch *et al.* 1980). This was also reported by (Stoyke *et al.* 1992) who identified sterile isolates, obtained from the roots of sub alpine Ericaceae plants, as *Oidiodendron griseum* Robak using RAPDS techniques. Unfortunately the vegetative morphology of the remaining sterile isolates in the present study was not distinctly similar to previously described genera, a requirement for further comparative work using molecular techniques.

The belated identification of sterile hyaline group 1 isolates as a species of *Phialophora* sp. was notable as this genus initially appeared to be absent from the surveyed Waikato pastures, despite being previously reported to be common on grass in New Zealand pastures (Thornton 1965, Skipp and Christensen 1989a). The absence of *Phialophora* from Waikato pastures was also reported by (Bonish 1989). *Phialophora radicicola* was readily detected in perennial ryegrass roots by both culture and microscopy (Skipp and Christensen 1989a) but can be difficult to isolate (Slope *et al.* 1978) and this may be why it was not recovered in this study. The species of *Phialophora* obtained in this study was isolated from clover (Appendix 2), differing from *P. radicicola* which has a host preference for ryegrass. Species of *Phialophora* have been associated with root disease of many gramineaceous plants (Deacon and Scott 1983, Smiley and Craven-Fowler 1984), however this species was non-pathogenic despite its ability to invade seedling and plant roots.

In culture sterile isolates do not produce any reproductive structures but most of the sterile groups here produced swollen thick walled chlamydospore-like cells, monilioid hyphae or closely aggregated strands of hyphae all of which could act as resting structures and allow long term survival in the soil, survival in adverse environmental conditions, and provide a source of inoculum for further infection. A parallel can be drawn between the aggregated hyphal strands produced by sterile fungi and the rhizomorphs of basidiomycetes. Thick walled cells and monilioid hyphae were often darkly pigmented and this dematiaceous pigmentation was another common characteristic which may also be important for the survival of these fungi.

Sterile fungi were one of the most important components of the Waikato pasture root mycoflora which suggests they have an important ecological role in pastoral rhizosphere. This study also found these isolates readily recolonised the epidermis and cortex of seedling and plant roots but were non pathogenic or mildly pathogenic to both legume and grass plants. Specialised non-parasitic, root-inhabiting fungi are often not very aggressive in damaging invaded tissue and are also characterised by a low degree of competitive saprophytic ability (Singh 1980). Many studies have also reported non-pathogenic sterile fungi to be important in the rhizosphere (Glynne 1939, Harley and Waid 1955, Waid 1957, Peterson 1958, Thornton 1958, 1965, Parkinson and Clarke 1961, Parkinson *et al.* 1963, Taylor and Parkinson 1965, Chu-Chou and Grace 1982, Hall 1987, Skipp and Christensen 1989a , Fisher *et al.* 1995a) and their high frequency of isolation from plant roots means they are typical and ubiquitous components of the mycoflora of higher plants (Parkinson and Pearson 1967). Six of the sterile groups studied here were slow growing, and previous studies report that slow growing sterile fungi occur relatively infrequently in roots and surrounding soils (Taylor and Parkinson 1961, Parkinson and Pearson 1967). In spite of this they were among the first fungi to colonise roots and penetrate all root tissues (Taylor

and Parkinson 1965) and, despite their physiological disadvantage of slow growth rates, these sterile fungi can compete effectively with rapidly growing fungi to be significant components of the pasture root mycoflora.

There have been various suggestions as to ecological significance and function of non- pathogenic sterile fungi. Some sterile dark fungi, in particular slow growing forms, have been reported to form mycorrhizal associations with plants (Singh 1965, 1974, 1980, Robertson 1975), and while there was no morphological evidence in this study that the sterile isolates were strict root endophytes or mycorrhizae this cannot be discounted. The conspicuous feature of the formation of swollen beaded cells and monilioid hyphae in the root cells of inoculated plants by several of these sterile groups are similar to the oidia produced by fungal root endophytes described by (Nicolson 1959), however the sterile isolates in this study differed, as swollen cells of most sterile groups were uninucleate, were not zygomycetous, and grew readily in culture. Structures which enable long term survival in soil may be more important than dispersal for many root dwelling fungi which may be an explanation for the high frequency of sterile fungi encountered in roots and soil, and why these fungi produce such structures rather than sporulate.

Waid (1957, 1974) reported that sterile fungi were part of a continuous succession of the root mycoflora and colonised aging and senescent roots. Old roots were systemically invaded by both hyaline and dark sterile fungi which suggests these fungi invade dying roots and assist the internal breakdown of root tissues (Taylor and Parkinson 1965, Hall 1985). Sterile fungi have previously been reported to breakdown simple carbohydrates as well as more complex carbohydrates such as lignin, pectin, hemicellulose and cellulose (Ross 1960), which would aid the decomposition of plant root material and increase their success as saprophytes, in both pastoral soils and aging roots. Sterile fungi, living in both roots and soil, which assimilate a large range of substrates possess the necessary enzymes to make use of simple and complex root materials and their predominance in a particular environment is not likely to be limited by nutritional factors (Ross 1960). In pastoral soils there are large quantities of plant roots and actively growing root fungi are capable of utilising senescent root materials (Ross 1960, Jackson 1965). This increased root decomposition is beneficial for the pastoral ecosystem in that root turnover rate and nutrient recycling is encouraged (Jackson 1965), and both these processes lead to increased plant production.

The sterile root-colonising fungi isolated from Waikato pasture roots are non pathogenic but actively penetrate pasture roots. It is therefore likely they have a similar ecological role to that previously reported for other sterile root-colonising fungi being saprophytes which promote the decomposition of senescent roots and thus beneficial to perennial pastures.

CHAPTER THREE - POTENTIAL PATHOGENICITY OF ROOT-COLONISNG FUNGI TO PASTURE LEGUMES AND GRASSES.

" one for the rat, one for the crow, one to rot, and one to grow"
anon

3.1 INTRODUCTION

In agricultural and pastoral soils, nutrient availability is a factor which limits microbial growth and activity (Nelson 1990). As a consequence most microorganisms, including pathogenic root fungi, exist in a state of exogenous dormancy or fungistasis (Lockwood 1977). Plant pathogenic fungi can survive in this quiescent state as resistant propagules, such as chlamydospores, which are produced on and in infected hosts during pathogenesis. The propagules are released into the soil upon decomposition of plant residues where they are then susceptible to fungistatic suppression (Lockwood 1988). Fungistasis has survival value for plant pathogens in soil as it prevents propagules from germinating in the absence of suitable hosts and dormant propagules are less vulnerable to antagonistic soil activity (Katan 1991). In order for root-pathogen interactions and fungal infection to be initiated the germination of these dormant propagules must be activated by stimulatory molecules present in seed and root exudates of susceptible hosts (Nelson 1990). Germinated propagules intercept host roots and produce infection structures, such as infection cushions, and enzymes to enable host tissue to be penetrated. The degree and type of pathogenesis or disease which develops after this initial infection will depend on the virulence of the pathogen, host susceptibility, environmental factors, and other biotic factors such as the surrounding soil microorganisms (Park 1963).

There are three main types of root disease caused by pathogenic soilborne fungi, damping-off, vascular wilt, and root rots. Visible symptoms of these diseases include necrosis, discoloration (pink, red, yellow, brown, and black), tissue destruction, gum formation, root distortion and malformation, stunting, and sloughed cortical cells (Kommedahl and Windels 1979). Symptoms are induced by the production of toxins or enzymes that predispose or kill cells in advance of penetration. In damping-off diseases in pastures occur when seedlings are infected and rotted rapidly at the pre- or post-emergence stages. *Rhizoctonia* and pythiaceous fungi can cause poor establishment of pasture and forage crops by killing emerging seedlings (Barbetti and MacNish 1978, Greenhalgh and Lucas 1984). Vascular wilt pathogens penetrate root tissues and systemically spread through the xylem vessels which become plugged and unable to conduct water (Katan 1991). Vascular wilt diseases of pastures are caused by species of *Fusarium* and *Verticillium* (Pratt 1982, Hawthorne 1983, Venuto *et al.* 1995). Root rot pathogens are usually necrotrophic fungi which kill root cells by active hyphal invasion and intracellular production of toxins. Other non-parasitic toxicogenic fungi do not infect host tissues but

induce disease by the extracellular production of toxins (Woltz 1978). Many genera of soilborne fungi cause serious root rots of pasture plant, these include *Cylindrocarpon* (Willis 1965, Skipp *et al.* 1986), *Cylindrocladium* (Nan *et al.* 1991a, Waipara *et al.* 1996c), *Fusarium* (Leath and Kendall 1978), and pythiaceous fungi (Stovold 1974). The pathogens causing damping-off, wilt or root rot are considered as major root pathogens because they cause visible shoot and root symptoms which may lead to plant death (Katan 1991).

There are also soilborne fungi which act as minor root pathogens (Salt 1979). Minor pathogens parasitise root tips, root hairs and superficial cortical root cells, causing little visible damage to plants but often having sustantial effects on root growth (Katan 1991) and nutrient and water uptake (Salt 1979). Minor pathogens also cause serious damage to roots in a synergistic combination with major or other minor root disease pathogens. For example, *Fusarium acuminatum* is a weak secondary pathogen of grass roots but can cause root rot in combination with *Fusarium culmorum* and *F. oxysporum* (Sprague 1950). In contrast to major pathogens, minor pathogens are more widely distributed in agricultural soils, have a wider host range, and are frequently isolated from apparently healthy roots (Salt 1979). The lack of distinctive disease symptoms has made determining the effects of these fungi and their effects in roots difficult.

Successful pathogens have evolved by the exploitation of the best opportunities for host infection. One way to achieve this is for inoculum to be transported with the seed of the host so infection can occur at the earliest opportunity and this also allows seedborne pathogens to be transported by hosts to new areas of cultivation (Richardson 1996). Seedborne infections by pathogens, such as *Fusarium oxysporum,* are transmitted to roots, vascular systems and cotyledons of germinated seedlings from contaminated seed coats or embryos (Maude 1996), and soilborne seed pathogens can also initiate infection from the soil. Seed colonisation by fungi is initiated when dormant seeds imbibe water and produce soluble or volatile exudates which stimulate growth of microbial populations on the seed coat and surrounding soil (Nelson 1990).

Failure of grass and legume seeds to establish in pasture seed beds can result from environmental stress, pest predation and diseases. Michail and Carr (1966) estimated that 60% or less of grass seed sown into field plots contributed plants to the final sward and that fungal pathogens, such as *Fusarium* and *Cylindrocarpon*, were the major contributory factors to seed and seedling mortality. Pathogenic soilborne fungi can affect the survival and emergence of both legume seed (Salonen 1972, Stovold 1975, MacNish 1977, Kellock *et al.* 1978, Omar *et al.* 1989, Barbetti 1984a, 1990), and grass seed (Holmes 1979, 1983, Falloon 1980b, 1981, Falloon and Fletcher 1985). Studies on the influence of fungi on pasture establishment has frequently been carried out by comparing emergence of plants from untreated seed with that from seed treated with a fungicide known to control particular seedling pathogens. Fungicide seed treatments of forage pasture plants have

improved the emergence, establishment and production yield of ryegrasses (Falloon 1980a, 1981, Falloon and Fletcher 1983, Falloon and Skipp 1982, Holmes 1983), cocksfoot and timothy (Michail and Carr 1966, Holmes 1979), tall fescue and meadow fescue (Holmes 1983), lucerne (Falloon and Skipp 1982), prairie grass *Bromus willdenowii* Kunth (Falloon 1985b) and subterranean clover (Greenhalgh and Clarke 1985). In some cases this application of fungicides has led to increased seed emergence of up to 100% in the field (Holmes 1983, Falloon 1985).

Pathogen-induced seed losses and decreased emergence may erode the long term sustainability of pastures. For example fungal disease losses of legumes in pastures when compounded annually can be sufficient to reduce seed reserves to below levels needed to maintain existing populations (Barbetti *et al.* 1996). This will reduce production and persistence of susceptible legumes until pastures become severely deteriorated and weedy with a low nitrogen levels. Regeneration and productivity of pasture plants can also be affected when root diseases reduce seed size, number and viability (Pratt 1989). Small seed sizes can produce weak and uncompetitive seedlings which further reduces plant persistence and yield (Black 1956).

The interactions of root pathogens with other soil microorganisms are major determinants of their survival, reproduction and pathogenesis (Katan 1991). Without the presence of other soil and root organisms which suppress disease, root pathogens could destroy all pasture plants and crops. Certain pastoral soils are naturally pathogen suppressive, as they reduce disease development (Schneider 1982). In contrast, other soils which are conducive to pathogens can enhance root disease. Disease suppressiveness has been attributed to natural biological control processes, such as mycoparasitism (Lumsden and Carroll 1992), antibiosis and competition (Katan 1991).

The interaction between a mixture of soilborne root pathogens and the host is complex. Zogg (1952) reported that fungal root pathogens of barley could act synergistically to cause more disease symptoms, but that a different mixture of pathogenic species reduced disease. This was also reported when mixed infections of wheat roots by the pathogen *Gaeumannomyces graminis* and the minor pathogen *Phialophora radicicola* decreased take-all damage (Deacon 1976). This is because multiple infections by a mixture of root pathogens can lead to competition or antagonism which results in biological control of root disease (Mangenot and Diem 1979).

The specific causes of root diseases are not always well defined as evidence of infections is not always obvious and can be confused with symptoms caused by abiotic stress factors (Grau 1996). Many of the disease problems in pastures also tend to occur at a subclinical level and may not be recognised (Flett and Clarke 1996). Root pathogens in perennial pastures interact, often synergistically, with environmental and other biotic

factors to cause a slow, steady decline of plant health and not a sudden and obvious epidemic as frequently witnessed for damping-off diseases (Grau 1996). This interactive mixture of fungal pathogens, other soil organisms, and abiotic factors, which combine to cause root disease is called a disease complex (Powell 1971, Flett and Clarke 1996, Grau 1996).

Most reports of fungal root rot diseases in New Zealand pastures document the mixed isolation of numerous pathogenic fungal species including species of *Fusarium, Verticillium, Rhizoctonia, Pythium, Cylindrocladium, Cylindrocarpon* and *Phoma* (Thornton 1965, Skipp and Christensen 1981,1982, 1989a, Skipp *et al.* 1986, Nan *et al.* 1991b). These fungi are common components of the pasture root mycoflora and have been associated with root rot disease of white clover, red clover, and perennial ryegrass. These same fungi have also been associated with root rot diseases of subterranean clover, white clover and lucerne in Australian pastures (Shipton 1967, Stovold and Wong 1973, MacNish 1977, Barbetti and MacNish 1978, Burnett *et al.* 1994a) and many grass and legume species in North American pastures (Kilpatrick *et al.* 1954a, 1954b, Kilpatrick 1958, Kilpatrick 1959, Kilpatrick and Dunn 1961, Halpin and McCarter 1961, Halpin 1963, Chi *et al.* 1964, Freter and Wilcoxson 1964, Willis 1965, Aube and Deschenes 1967, Cressman 1967, Johnson and Keeling 1969, Kreutzer 1972, Campbell 1980, Farr *et al.* 1989).

Fungal root disease are important causes of declining productivity of plants in both irrigated and dryland pastures in Australia, particularly those containing subterranean clover and lucerne (Ludbrook *et al.* 1953, Burgess *et al.* 1973, Stovold and Wong 1973, Stovold 1974, Wong 1975, Wong *et al.* 1985a, 1985b, Taylor and Greenhalgh 1987). A wide range of pathogenic fungi have been isolated from root rot lesions from Australian pastures which led to the conclusion these fungi are the cause of decline and poor re-establishment of introduced pasture plants (Johnstone and Barbetti 1987).

Disease complexes of fungal pathogens and nematode species are widely reported in Australasian pastures (Skipp and Watson 1987, 1996, Flett and Clarke 1996) where the presence of nematodes on roots increased the root colonisation by fungi such as *Fusarium.* The combined effects of root pathogenic fungi and nematodes have been reported to be greater than the damage caused by fungal pathogens alone (Halpin 1963) because nematode attack of roots enhances entry by root-rot fungi into root tissues (Dropkin 1979). Root and foliage feeding by other herbivorous insects also increased root-rot severity and fungal colonisation of pathogenic *Fusarium* species in red clover pastures (Leath and Byers 1977, Hatcher 1995). *Fusarium* vascular wilts in pastures of alsike clover (*Trifolium hybridum* L.) increased after insect damage by *Sitona hispidula* Fabr. (Leach *et al.* 1963). Pasture plant disease complexes between pathogenic root fungi and bacteria, viruses and other foliage diseases have also been reported to reduce pasture production (Grau 1996).

Pasture plants are susceptible to pathogenic fungi of all major taxonomic classes (Grau 1996, Skipp and Hampton 1996, Skipp and Watson 1996). Over 40 fungal diseases have been recorded on grasses and forage legumes in New Zealand pastures (Pennycook 1989, Skipp and Hampton 1996), however, most of these have been foliage pathogens which cause conspicuous disease symptoms.

Foliage diseases in New Zealand pastures include rusts (*Puccinia* spp. and *Uromyces* spp.), anthracnose (*Colletotrichum graminicola* (Ces.) Wilson), smuts (*Ustilago* spp.), mildews (*Erysiphe* spp., *Sclerophthora* sp.), scorch (*Aureobasidium caulivora* Kirchner) and numerous types of leaf spot and blotch diseases which are caused by many different species of fungi. These diseases act as constraints to pasture production. For example, rust epidemics on pasture grasses reduced shoot and root weights, restricted plant growth, increased grass tiller senescence and decreased seed production (Skipp and Hampton 1996). The subterranean clover foliage pathogen *Myrothecium verrucaria* has also been associated with root rots of several forage legumes (Leath and Kendall 1983, Barbetti 1984b) showing that systemic infections by foliage pathogens can spread to cause secondary root diseases in pastures. In New Zealand there are few pasture root diseases reported to be caused by a single causal organism (Chakraborty *et al.* 1996), however root-rots caused by *Sclerotinia* spp., *Phytophthora* spp., *Pythium* spp. and *Staganospora* have been reported (Skipp and Hampton 1996).

Abiotic or environmental stresses in soils may influence the severity of root rot pathogens in pastures. Damage caused by oomycete root pathogens increased in pastures susceptible to flooding or soggy soil syndrome (Holub and Grau 1989, Grau 1996). Temperature has also been reported to affect pathogenic root fungi. Injury due to cold temperatures and frost has been implicated in pasture decline (Grau 1996) as plants are then more susceptible to invasion by psycrophilic fungi such as *Fusarium nivale*, a root pathogen of grasses, which is active at 5°C (Colhoun 1979). In contrast the clover root rot pathogen *Codinaea fertilis* is more active at high temperatures (Campbell 1982).

It is the progressive interaction of these disease complexes and environmental factors which results in a cumulative stress load which can combine to degrade the health of individual plants or entire pastures, leading to lower pasture productivity (Grau 1996)

Diseases not only cause significant losses in pastures, and as a consequence decrease the quality and quantity of forage plants, but also affect crops grown in rotation with pastures (Barbetti *et al.* 1996). Pathogen induced losses can be direct or indirect, direct losses including reduced plant growth measured as decreased herbage, diminished nutritional value (e.g., reduced protein, carbohydrate, dry matter digestibility), palatability, seed set and viability, and increased production of detrimental metabolites and mycotoxins.

Indirect losses range from undesirable changes in botanical composition of pastures (Lancashire and Latch 1970) to reduced nitrogen fixation inputs from legumes into pastures (Leath *et al.* 1971).

New Zealand derives over 50% of its export income from grassland-based products (Chakraborty *et al.* 1996), and diseases are a constraint to productivity, persistence and quality of pastures. Pastures are sown with a wide range of annual and perennial plants, and worldwide there have been over 400 fungal, bacterial, viral, mycoplasma and nematode diseases reported to affect forage grass and legume plants (Haggar *et al.* 1984). These pathogens combined, are a constraint to increased plant production, and have been estimated to cause a 10% loss in current pasture production (Chakraborty *et al.* 1996).

Nitrogen (N) is a major limiting growth factor for pasture plants (Heichel 1987). In New Zealand pastures N deficiency is the major limitation to plant production (Steel 1982) and current pasture production could be increased by 30% by the correction of N deficiency. Nitrogen supply for pasture growth is dependent on fixation by the symbiotic relationship between *Rhizobium* spp. and white clover which increases the fertility status of the soil enabling the growth of highly productive grass species such as perennial ryegrass. White clover has the potential capacity to fix up to 500 kg /N /ha /yr (Sears *et al.* 1965), and in New Zealand pastures averaged 180 kg / N / ha / yr (Hoglund *et al.* 1979). The annual financial contribution of white clover in New Zealand through fixed nitrogen, forage yield and seed production is estimated at $3.1 billion (Caradus *et al.* 1995) with most of this due to the estimated 1.57 million tonnes of N is fixed annually. Clover production losses caused by fungal root rot can therefore be calculated on the cost of applying N based fertilisers (urea) to replace the reduced inputs of nitrogen attributable to disease.

Clover is an important component of all pasture swards in New Zealand and the influence of root-colonising fungi, particularly soilborne pathogens, on the persistence and health of clover is of agricultural importance. It is therefore of concern that the disappearance of clover from perennial North Island pastures has been reported (Menzies 1973a, Skipp and Watson 1987, 1996). This problem, described as 'clover decline' or 'clover collapse', has indirectly been attributed to soilborne pathogenic fungi and nematodes, as the treatment of pasture swards with fungicides and nematicides has increased pasture production, clover vigor and N fixation (Watson *et al.* 1985, Skipp and Watson 1987, Nan *et al.* 1992). Widespread pasture decline has been reported elsewhere (Labruyere 1979, Hochman *et al.* 1990, Burnett *et al.* 1994b), and fungal root diseases in combination with other biotic and abiotic factors have contributed to this (Flett and Clarke 1996). By affecting roots, fungal pathogens can reduce productivity and persistence by directly reducing size and numbers of *Rhizobium* nodules on legume plants and fungal infection can also impair nodule function (Barbetti *et al.* 1996). Further research is

required to elucidate the interactions and extent to which fungal pathogens cause root disease and production loss of clovers and other plants in New Zealand pastures.

If problems in pasture health are understood, then vast areas of grazing and forage cropland could be better utilised for plant and animal production from more sustainable pastures. Persistent legume-based pastures help conserve soil, water and land resources, enhance soil fertility and increase plant and animal production (Chakraborty *et al.* 1996).

This third section of work examined the pathogenicity of root-colonising fungi to seed, seedlings and plants of common Waikato pasture grasses and legumes. This may also enable the proportion and diversity of fungal pathogens present in roots to be estimated. This section particularly focused on the pathogenicity of root-colonising fungi to white clover, as it was important to determine if these contribute to the persistence problems of this vital plant in perennial pastures throughout the North Island.

3.2 MATERIALS AND METHODS

3.2.1 PATHOGENICITY OF ROOT FUNGI TO AXENICALLY GROWN SEEDLINGS.

3.2.1. (a) An *in vitro* pathogenicity Petri plate test.

With over 121 fungal species being isolated from pasture roots, it was important to ascertain their pathogenicity and root colonisation. Seventy fungal species (Table 3.1) were screened against 12 pasture species to assess root-colonisation. All 70 isolates are part of the Canterbury University microbial culture collection and are stored in 10% glycerol at -80°C. These isolates are also stored on PCA slopes as living cultures, as part of the EPM microfungi collection at AgResearch, Ruakura Agricultural Research Centre.

Table 3.1 Fungal isolates screened against twelve pasture species.

Fungal species	Strain number	Original host
Acremoniella atra	C1WN5P7S2B	White clover
Acremonium curvulum	SV1WS7P8S16	Sweet vernal
Acremonium strictum	R3R4.2P5S1	Ryegrass
Arthrinium arundinis	C1WS1.1P8S1	White clover
Aspergillus niger	C1WS11P1S11	White clover
Aspergillus ustus	R1R6.2P6S7	Ryegrass
Bimuria novae zelandiae	C1R6.1P28S5	White clover
Botrytis cinerea	B2WS2P1S8	Browntop
Chaetomium globosum	C2R4.2P7S6	White clover
Chaetomium funicola	R1WN4.2P2S1	Ryegrass
Clasterosporium	SV1WN3P15S3	Sweet vernal
Codinaea fertilis	C1R6.1P2S5C	White clover
Colletotrichum sp.	C1WS11P8S7	White clover
Curvularia trifolii	R1WS3P3S8B	Ryegrass
Cylindrocarpon destructans	R1R6.2P17S1	Ryegrass
Cylindrocladium scoparium	C2R4.2P15S15	White clover
Dactylaria acerosa	C1WN4P3S8	White clover
Dreschlera dematioidea	SV2WSB1P5S1	Sweet vernal
Fusarium acuminatum	SV1WN3P2S1	Sweet vernal
Fusarium avenaceum	SVWNA2P11S2	Sweet vernal
Fusarium crookwellense	C2WNB1P4S2	White clover
Fusarium culmorum	R1R4.3P21S7	Ryegrass
Fusarium equiseti	R1WSB1P17S4	Ryegrass
Fusarium graminum	SV1WS3P7S5	Sweet vernal
Fusarium oxysporum	R2WNA1P5S7	Ryegrass
Fusarium sambucinum	SV1WS4P6S2B	Sweet vernal
Fusarium solani	R1R4.2P12S12	Ryegrass
Fusarium tricinctum	SV2WS7P6S1	Sweet vernal
Gliocladium roseum	C2WNA3P4S3	White clover
Gongronella butleri	R2R4.2P7S1	Ryegrass
Idriella bolleyi	R2WNA3P19S5	Ryegrass
Mariannaea elegans	R1R6.2P6S2	Ryegrass
Metarhizium anisopliae	SV2WNA2P7S4	Sweet vernal
Mortierella alpina	R1R4.2P4S8	Ryegrass
Mortierella elongata	B1WN2.2P3S7	Browntop
Mortierella gamsii	R2WS3P1S1	Ryegrass
Mortierella globulifera	B1WS2R2P8S3	Browntop
Myrothecium verrucaria	C2WNA2P21S4	White clover
Paecilomyces carneus	SV2WSBP10S1C	Sweet vernal
Paecilomyces lilacinus	SV2WNB1P11S3	Sweet vernal
Paecilomyces marquandii	R2R4.2P1S3	Ryegrass

Fungal species	Strain	Original host
Penicillium brevicompactum	C1R4.3P4S3	White clover
Penicillium chrysogenum	R1R4.2P13S7	Ryegrass
Penicillium decumbens	B1WN2P6S6	Browntop
Penicillium griseofulvum	R1R4.3P11S2	Ryegrass
Penicillium janczewskii	R1WN4P1S2	Ryegrass
Penicillium janthinellum	SV2WNA2P2S1	Sweet vernal
Penicillium oxalicum	L3WSP1S2	Lotus
Penicillium simplicissimum	B2WNB1P6S5	Browntop
Penicillium variabile	B1N1P2S3	Browntop
Periconia macrospinosa	SVWNA1P4S1	Sweet vernal
Phymatotrichum omnivorum	R1R4.2P6S10	Ryegrass
Pithomyces chartarum	B2WS10P1S2	Browntop
Plectosporium tabacinum	R2WNB1P2S3	Ryegrass
Preussia aemulans	SV1WS4P1S1	Sweet vernal
Ramichloridium schultzeri	C2WS2P7S7	White clover
Rhizoctonia solani	R1WSP11S2A	Ryegrass
Sordaria fimicola	R2R6.1S19S2	Ryegrass
Tetraploa aristata	C1R6.1P27S5	White clover
Thielaviopsis basicola	C1R4.3P10S12	White clover
Thozetella tocklaiensis	B2WNA2P2S4	Browntop
Tricellula sp.	SCWS5P2S5	Subterranean clover
Trichoderma hamatum	R2R4.3P12S1	Ryegrass
Trichoderma harzianum	C2R6.2P3S3	White clover
Trichoderma koningii	C2WNB1P10S1A	White clover
Trichoderma polysporum	C2R4.3P8S2	White clover
Trichoderma viride	R2R6.2P10S2	Ryegrass
Trichosporon cutaneum	C1R4.3P25S5	White clover
Verticicladiella sp.	R2R4.2P1S6	Ryegrass
Verticillium chlamydosporium	SVWNA3P14S5	Sweet vernal

A single isolate of each fungus was used in this screen but a preliminary assessment of the strain variation between isolates of the same fungus was undertaken, and these results are presented in Appendix 7.

Eight of the plant species had been used in the survey of Chapter 1, and the four additional plants used; tall fescue, cocksfoot, timothy and red clover are common in Waikato pasture swards (Table 3.2). To maximise consistency of results fresh seed of each plant were obtained from the Margot Forde Germplasm Centre, Agresearch Grasslands, Palmerston North.

Table 3.2 Twelve plant species screened *in vitro* for susceptibility to 70 fungal species.

Common name	Species name	Grasslands cultivar
White clover	*Trifolium repens* L.	Huia
Lotus	*Lotus uliginosus* Schkuhr	Maku
Subterranean clover	*Trifolium subterraneum* L.	AK1003
Red clover	*Trifolium pratense* L.	Pawera
Timothy	*Phleum pratense* L.	Kahu
Yorkshire fog	*Holcus lanatus* L.	Massey Basyn
Browntop	*Agrostis capillaris* L.	Egmont
Sweet vernal	*Anthoxanthum odoratum* L.	BZ 2330
Tall fescue	*Festuca arundinacea* L.	Roa
Cocksfoot	*Dactylis glomerata* L.	Wana
Perennial ryegrass	*Lolium perenne* L.	Nui endophyte-free
Soft brome (Goosegrass)	*Bromus hordeaceus* L. (= *B. mollis* L.)	Whatawhata wild type

A rapid *in vitro* screening technique was used to determine pathogenicity and root colonisation of each fungus (Christensen *et al.* 1988). Seeds were surface-sterilised in 1% sodium hypochlorite solution for 5 minutes, followed by three rinses in sterile water. Sweet vernal, soft brome, red clover and subterranean clover seeds were also soaked in 50% sulphuric acid for 15 min as a preliminary treatment, as they had rough, hairy or hard seedcoats. Seeds were germinated on 1.5% WA in Petri plates and incubated at 20°C. Surface-sterilised seeds were all placed in a line across each WA plate. Plates were taped together in groups and aligned horizontally so that seeds would germinate vertically across the plates (Figure 3.1). After a minimum of seven days, seedlings were inoculated with 5mm PCA culture plugs of fungus (Figure 3.2). Inoculated plates for each fungus were examined 10 days after inoculation and scored for root disease symptoms. A disease score 0-5 was assigned to each seedling based on observable disease symptoms. The following disease scores were used; 0 = white turgid roots with no visible symptoms; 1 = light discoloration (yellowing or browning) of root tissue; 2 = dark brown discoloration of root tissue and inhibition of root growth; 3 = root surface lesions present and inhibition of root growth; 4 = systemic root lesions and necrosis; 5 = complete seedling death. A mean disease score was then calculated for each host by averaging all six disease scores for each host plant. Fungi with a mean disease score >4 were classed as being pathogenic, fungi with scores between 3-3.9 were classed as mildly pathogenic, fungi with scores between 2-2.9 were classed as weakly pathogenic and fungi with scores <2 were classed as non-pathogenic. A root segment from each inoculated seedling was plated onto WA to reisolate the fungus from diseased tissue. A pathogenicity rating was calculated for each isolate on each host by averaging the six disease scores from plates for each treatment.

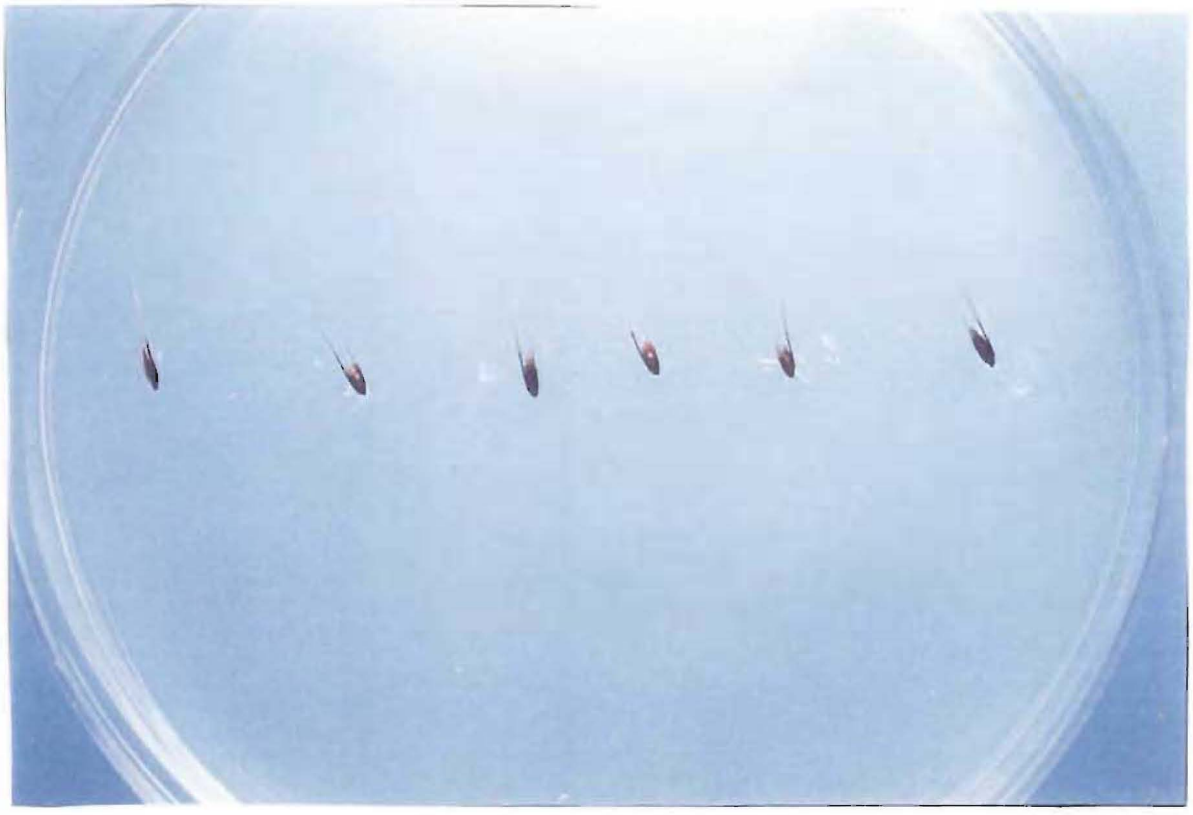


Figure 3.1 Horizontal plating of six surface-sterilised sweet vernal seeds on water agar.

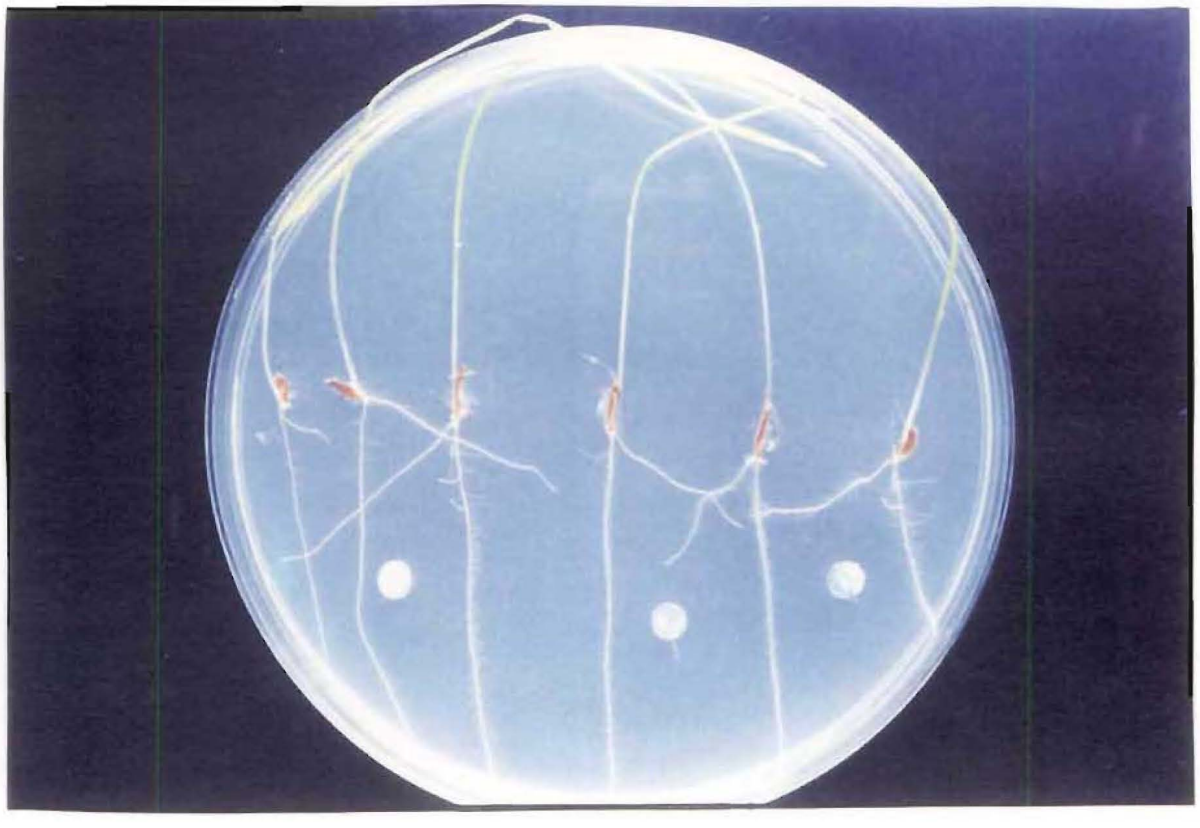


Figure 3.2 Germinated soft brome seedlings inoculated with fungal plugs.

3.2.1 (b) Staining method to assess fungal colonisation.

Hyphal penetration of colonised seedlings was determined microscopically. Twenty longitudinal and transverse sectioned root segments (1 mm thick) were cleared in 10% KOH at 60°C for 4 hr, rinsed in 10% H_2SO_4 and stained with 5% trypan blue in lactophenol at 60°C for 45 min. Root sections were then mounted on a slide in a drop of lactophenol and examined by light microscopy. Roots were rated on the extent of fungal hyphal colonisation. The following colonisation scores were used; 0 = hyphal penetration absent; 1 = epidermal colonisation; 2 = cortex colonisation; 3 = inner cortex and vascular tissue colonisation.

3.2.1.(c) Light and Transmission Electron Microscopy (TEM) of infected root tissue.

Sections of inoculated seedling roots were prepared for Transmission Electron Microscopy (TEM) to observe fungal colonisation of infected root cells but only a sample of infected seedlings were examined by TEM. Root segments of ryegrass, subterranean clover and white clover were sectioned 12 days after being inoculated with a sample of eight pathogenic, and two mildly or non-pathogenic fungi (Table 3.3). Inoculated subterranean clover seedlings were used in all the TEM samples as they had larger roots which remained undamaged after sectioning and TEM preparation methods outlined below. Infected white clover and ryegrass roots were also sectioned.

Table 3.3 Fungi inoculated onto plant hosts for examination by TEM.

Fungus	Host plant
Pathogens:	
Codinaea fertilis	WC*, SC, RG
Cylindrocarpon destructans	SC
Cylindrocladium scoparium	WC, SC
Fusarium crookwellense	WC, SC, RG
Fusarium culmorum	WC
Mortierella gamsii	SC
Myrothecium verrucaria	WC
Non/weak pathogens:	
Bimuria novae zealandiae	SC
Sterile dark group 6	SC

*WC white clover, SC subterranean clover, RG ryegrass.

Transverse sections (50 μm) of infected seedling roots were fixed in 2.5% gluteralderhyde in 0.075M Na-Na phosphate buffer (pH =7.2), at 20°C. Sections were removed from the fixative after 2 hr and placed in 1% osmium (O_sO_4) in 0.025M Na-Na phosphate buffer (pH =7.2) at 20°C for a 3 hr post fixation buffering. Sections were then chemically dehydrated by soaking them in 20, 40, 60, 80% acetone for 10 minutes each, followed by three 10 minute changes in 100% acetone. Each section was then infiltrated with 30% LR White acrylic resin in 100% acetone for 12 hr, and then embedded in 100% LR White resin, polymerised at 70°C. The sections were left to set in the resin overnight,

after which the resin blocks were trimmed and sectioned further by an Ultramicrotome (LKB Ultrotome IV). Sectioned root segments were examined by light microscopy before being set onto a heated microscope slide and post-stained with uranyl acetate-lead citrate. Following this post-staining, the sections were examined using a Hitachi HS-75 transmission electron microscope.

3.2.2 EFFECT OF ROOT-COLONISING FUNGI ON THE EMERGENCE OF PASTURE SPECIES

An experiment was undertaken to assess the capacity of these fungi as seed pathogens. Twenty nine fungi (Table 3.4) were tested for their effect on the seedling germination of pasture plants. Most of the fungi selected for this test were shown to be pathogenic to axenically grown seedlings outlined previously, and four sterile fungi were also tested.

Table 3.4 Fungi inoculated onto the seeds of twelve pasture species.

Fungal Species	Strain	Original host
Acremonium strictum	R3R4.2P4S1	White clover
Bimuria novae zelandiae	CIR6.1P28S5	White clover
Botrytis cinerea	B2WS2P1S8	Browntop
Codinaea fertilis	C1R6.1P2S5C	White clover
Cylindrocarpon destructans	R1R6.2P17S1	Ryegrass
Cylindrocladium scoparium	C2R4.2P15S15	White clover
Dreschlera dematioidea	SV2WSB1P5S1	Sweet vernal
Fusarium acuminatum	SV1WN3P2S1	Sweet vernal
Fusarium avenaceum	SVWNA2P11S2	Sweet vernal
Fusarium crookwellense	C2WNB1P4S2	White clover
Fusarium culmorum	R1R4.3P21S7	Ryegrass
Fusarium equiseti	R1WSB1P17S4	Ryegrass
Fusarium oxysporum	R2WNA1P5S7	Ryegrass
Fusarium sambucinum	SV1WS4P6S2B	Sweet vernal
Fusarium solani	R1R4.2P12S12	Ryegrass
Fusarium tricinctum	SV2WS7P6S1	Sweet vernal
Mortierella gamsii	R2WS3P1S1	Ryegrass
Myrothecium verrucaria	C2WNA2P21S4	White clover
Phymatotrichum omnivorum	R1R4.2P6S10	Ryegrass
Plectosporium tabacinum	R2WNB1P2S3	Ryegrass
Rhizoctonia solani	R1WS1P11S2A	Ryegrass
Sterile dark fungi group 1	RIR6.1P2S7	Ryegrass
Sterile dark fungi group 5	C1WN5P9S7	White clover
Sterile dark fungi group 6	R2WS7P10S7	Ryegrass
Sterile hyaline fungi group 1	SV2WNB1P1S2	Sweet vernal
Thielaviopsis basicola	CIR4.3P10S12	White clover
Trichoderma hamatum	R2R4.3P12S1	Ryegrass
Trichoderma harzianum	C2R6.2P3S3	White clover
Trichoderma koningii	C2WNB1P10S1A	White clover

Fungi were inoculated into pots sown with seeds of twelve of the plant species listed (Table 3.2), using the same fungal isolates as in the previous experiment. Fifteen 5mm fungal plugs from the margins of seven day old PCA cultures were placed in pots containing 80g of sterile sand. The plugs were covered with 10 mm depth of sand and watered with 30 ml of sterile distilled water. Host seed was surface-sterilised in 50%

H_2SO_4 for 20 minutes and 1% NaOCl for 5 minutes. Ten seeds were planted into each pot above the inoculum. Pots were maintained at 20°C for 2 weeks, after which seedling emergence was assessed. Control pots were inoculated with sterile PCA plugs and each treatment was replicated three times. After 2 weeks the numbers of seedlings per pot were counted. Seedlings were harvested from each pot and washed to remove sand particles. A disease score 0-5 was assigned to each seedling based on observable disease symptoms. The following disease scores were used; 0 = white turgid roots with no visible symptoms; 1 = light discoloration (yellowing or browning) of root tissue; 2 = dark brown discoloration of root tissue and inhibition of root growth; 3 = root surface lesions present and inhibition of root growth; 4 = systemic root lesions and necrosis; 5 = complete seedling death. A mean disease score was then calculated for each host by averaging all disease scores for each host plant. Any seed or seedlings with disease symptoms were also surface-sterilised and plated onto WA to reisolate the inoculated fungus.

3.2.3 EFFECT OF ROOT FUNGI ON THE YIELD OF SHOOT AND ROOTS OF PASTURE PLANTS

The potential loss of plant productivity caused by fungal pathogens was determined in pot trials where pasture plants grown in sterilised and unsterilised soil were inoculated with pathogenic fungal species and the dry weight yield determined after four weeks growth. The yields were statistically compared to the yields of uninoculated control plants, as well as plants inoculated with non-pathogenic fungal species.

3.2.3. (a) Inoculation of plants grown in fumigated soil

Cultivars of six pasture plants; white clover (Huia), perennial ryegrass (endophyte-free Nui), perennial ryegrass (endophyte *Acremonuim lolii*-infected Nui), browntop (Egmont), sweet vernal (BZ2330) and soft brome (Whatawhata WT) were grown in pots and inoculated separately with 30 fungal species to assess the effect of fungi on shoot and root weight. The pasture plants selected for this trial were the four dominant plant species from surveys one and two, with the two of contrasting endophyte status chosen to determine whether the presence of endophyte influences the effect of root fungi on plant weights. Soft brome was included as it appeared relatively resistant to fungal root pathogens in the previous *in vitro* pathogenicity screen.

Plants were grown from surface-sterilised seed in Horotiu sandy loam soil (pH 5.6) which had been crushed through a 2 mm diameter sieve, placed in a desiccator and sterilised by fumigation with chloroform vapour (Jenkinson and Powlson 1976a). Soil was kept at 70-80 % water holding capacity (WHC) for the duration of the trial. The WHC of the soil was determined by a method outlined by (Jenkinson and Powlson 1976b), where 50 ml of water was added to 50g of dried soil in a clipped conical filter funnel. After 30 minutes, the funnel was unclipped and the volume of water draining in 30 minutes was

measured. The difference between the volume of water added and the volume of water drained, is taken as the WHC.

Seeds were planted into plastic pots (73 mm x 45 mm diam.) and once germinated, five plants per pot were left to grow for four weeks. A Rhizobium inoculum of 1 ml of $1x10^9$ cells of *Rhizobium leguminosarum* biovar *trifolii* Dangeard (Strain 2163-89 from the International Collection of Micro-organisms from Plants, PDD Herbarium, Landcare Research, Auckland, New Zealand) was added to all pots, one week after seed germination. This treatment was to ensure nodulation of clover for the trial but was added to all host plants to ensure uniform treatment for each pot.

Eighteen fungi were selected to be inoculated onto the plant hosts and these are listed in Table 3.5

Table 3.5 Fungi screened against twelve pasture hosts.

Fungal species	Strain	Original host
PATHOGENS		
Codinaea fertilis	C1R6.1P2S5C	White clover
Cylindrocarpon destructans	R1R6.2P17S1	Ryegrass
Cylindrocladium scoparium	C2R4.2P15S15	White clover
Fusarium acuminatum	SV1WN3P2S1	Sweet vernal
Fusarium avenaceum	SVWNA2P11S2	Sweet vernal
Fusarium crookwellense	C2WNB1P4S2	White clover
Fusarium culmorum	R1R4.3P21S7	Ryegrass
Fusarium equiseti	R1WSB1P17S4	Ryegrass
Fusarium oxysporum	R2WNA1P5S7	Ryegrass
Fusarium sambucinum	SV1WS4P6S2B	Sweet vernal
Fusarium solani	R1R4.2P12S12	Ryegrass
Fusarium tricinctum	SV2WS7P6S1	Sweet vernal
Myrothecium verrucaria	C2WNA2P21S4	White clover
NON PATHOGENS		
Gongronella butleri	R2R4.2P7S1	Ryegrass
Mariannaea elegans	R1R6.2P6S2	Ryegrass
Paecilomyces carneus	SV2WSBP10S1C	Sweet vernal
Thozetella tocklaiensis	B2WNA2P2S4	Browntop
Verticillium chlamydosporium	SVWNA3P14S5	Sweet vernal

Fungal inocula were prepared by inoculating culture plugs of each fungus into 600 ml bottles containing 150 ml of sloped PCA. Bottles were incubated at 20°C for 21 days or until cultures were sporulating copiously and spore suspensions obtained by scraping conidia into SDW. Test plants were inoculated by adding 15 ml of spore suspension ($1x10^7$ conidia/ml) into each pot. The suspension was pipetted into the soil up to a 30 mm depth (measured with a marked pipette tip), and pots were inoculated twice, 3 days apart, to ensure dissemination of conidia through the soil. Control plants were inoculated with 15 ml of sterilised (autoclaved for 20 min at 120°C) spore suspensions.

Plants were maintained for 5 weeks under artificial illumination (110 μ Einsteins m^{-2} sec^{-1}) in a controlled environment room at 20°C with 16 hr light and 8 hr dark periods. Plants were watered by weight periodically to maintain the correct WHC. Pots were replicated 3 times per treatment and were randomly arranged in the growth room. Each pot

was placed in a second outer pot to prevent cross contamination of spore suspensions through watering.

Plants were harvested five weeks after inoculation. Roots were washed and visually assessed for percentage root area affected by root rot. Each plant was assigned a root disease score 0-5 as follows: 0= Lesions and necrosis absent from roots; 1= 1-20% of total root area necrotic; 2= 21-40% of total root area necrotic; 3= 41-60% of total root area necrotic; 4= 61- 80% of total root area necrotic; 5= 81-100% of total root area necrotic.

Ten surface sterilized root segments from each plant inoculated with each fungal species were plated onto WA to reisolate each of the inoculated fungi. All remaining roots were detached from shoot material, then root and shoot components were placed in separate paper bags, and dried in a 60°C oven for 48 hr to determine a dry weight yields.

3.2.3. (b) Inoculation of plants grown in non-fumigated soil.

Potential pathogenicity was tested in a smaller pot trial by inoculating plants of white clover, sweet vernal, browntop and perennial ryegrass (endophyte free), grown in non-fumigated soil, with nine pathogenic fungi (Table 3.6). The methods were the same as for the pot trial above, except that soil was frozen for 1 hr to -20°C and maintained at this temperature for seven days to kill nematodes but retain other elements of the soil biota. Dry weights of roots and shoots of plants and root disease symptoms were again assessed after five weeks growth. Ten surface-sterilised root segments from each plant of each species were plated onto WA to reisolate the inoculated fungi.

Table 3.6 Fungal pathogens screened against four pasture hosts.

Fungal species	Strain	Original host
PATHOGENS		
Codinaea fertilis	C1R6.1P2S5C	White clover
Cylindrocarpon destructans	R1R6.2P17S1	Ryegrass
Cylindrocladium scoparium	C2R4.2P15S15	White clover
Fusarium avenaceum	SVWNA2P11S2	Sweet vernal
Fusarium crookwellense	C2WNB1P4S2	White clover
Fusarium culmorum	R1R4.3P21S7	Ryegrass
Fusarium solani	R1R4.2P12S12	Ryegrass
Fusarium tricinctum	SV2WS7P6S1	Sweet vernal
Myrothecium verrucaria	C2WNA2P21S4	White clover

Statistical analysis: All data were statistically analysed using analysis of variance, and LSD tests were used for mean separation.

3.2.4 EFFECT OF MOISTURE AND TEMPERATURE ON PATHOGENICITY OF ROOT PATHOGENS OF WHITE CLOVER.

Two common environmental factors which influence plant root growth in pastures are moisture and temperature. Roots respond to edaphic stresses such as moisture deficit and pot trials were therefore undertaken to assess pathogenicity of three fungal root

pathogens to white clover plants, grown under different soil moisture and temperature regimes. The three pathogens selected from previous experiments were, *Codinaea fertilis, Cylindrocladium scoparium* and *Fusarium crookwellense.*

3.2.4.1 *Codinaea fertilis*

Clover seeds were sown into pots (five per pot) containing 70 g of fumigated Horotiu sandy loam soil. Pots were then watered to four levels of soil moisture content; 40%, 50%, 60% and 80% WHC using the previously described method. Five week old plants were inoculated with two strains of *C. fertilis,* strain #1 (Cod C1R6.1P5S5C) having been isolated from white clover roots and strain #2 (Cod R2R6.1) from perennial ryegrass roots. Plants in each pot were inoculated with 15 ml of conidial suspensions (10^7 conidia/ml), produced by growing each strain on PCA at 25°C for 3 weeks. The suspension was pipetted into the soil of each pot up to a depth of 30 mm (measured with a marked pipette tip), and pots were inoculated twice, 3 days apart, to ensure dissemination of conidia through the soil. Control plants were inoculated with sterilised conidial suspensions. Plants were grown at 20°C for a further 4 weeks under artificial illumination (110 μ E m^{-2} sec^{-1}) with a 16 hr light and 8 hr dark photoperiod and were watered by weight periodically to maintain the four moisture regimes. Each soil moisture treatment was replicated five times for each strain and the control.

After 4 weeks growth, plants were removed and washed free of soil. Roots were visually assessed for percentage root area that was affected by root rot and each plant was assigned a root disease score 0-5 as described for the previous pot experiments. Surface-sterilised 3 mm root segments (10 from each plant) were plated onto water agar to reisolate *C. fertilis* at the conclusion of the experiment. Root and shoot components were then dried in an oven for 48 hr at 60°C to determine dry weight yields.

3.2.4.2 *Cylindrocladium scoparium*

Clover seeds were sown into pots (five per pot) containing 70 g of fumigated Horotiu sandy loam soil. Pots were watered to four levels of soil moisture content; 40%, 50%, 60% and 80% WHC placed into a metal grill inside one of three water baths. The water baths were set at three separate temperatures, 15°C, 20°C and 25°C. Five week old plants were inoculated with one strain of *C. scoparium* (C2R4.2P15S15) which was isolated from white clover roots. Plants in each pot were inoculated with 15 ml of conidial suspensions (10^7 conidia/ml), produced by growing each strain on PCA at 25°C for 3 weeks. The suspension was pipetted into the soil of each pot up to a depth of 30 mm (measured with a marked pipette tip), and pots were inoculated twice, 3 days apart, to ensure dissemination of conidia through the soil. Control plants were inoculated with sterilised conidial suspensions. Plants were grown at 20°C for a further 4 weeks under artificial illumination (110 μ E m^{-2} sec^{-1}) with a 16 hr light and 8 hr dark photoperiod and were watered by

weight periodically to maintain the four moisture regimes. Each soil moisture treatment was replicated five times for each strain and the control.

3.2.4.3. *Fusarium crookwellense*

The same experimental design and methods used to assess the pathogenicity of *C. scoparium* were again applied to inoculate *F. crookwellense* to white clover, at the same three levels of soil moisture (40%, 60%, 80%) and temperatures (15°C, 20°C, 25°C).

All data from these pot experiments were analysed by analysis of variance using LSD tests for mean separation.

3.2.5 *IN SITU* EXAMINATION OF ROOTS INOCULATED WITH ROOT PATHOGENS USING A MINIRHIZOTRON-BORESCOPE SYSTEM.

The study of soilborne root pathogens has been hampered by the difficulty in making *in situ* observations on root systems obscured by the soil matrix (Rush *et al.* 1984). Development of the minirhizotron-borescope system facilitates observation of root growth and distribution (Bohm 1979). This method uses minirhizotron tubes placed in soil with a window to the root system provided by a borescope camera which is inserted into the tubes so that roots intersecting the tubes can be directly observed (Upchurch and Ritchie 1983, Taylor 1987). The clear acrylic observation tubes which allow the extendable camera to be inserted into the soil are illuminated by fiber optic light guides. This method provides a technique for root observation in a natural environment which can be used repeatedly over a continuous time period (Upchurch and Ritchie 1983).

This apparatus was developed for use in agronomy to quantify the dynamics of root growth and water uptake of important crops. However, Rush *et al.* (1984) have applied this system for *in situ* observation of *Phymatotrichum omnivorum,* an important phytophagous root pathogen of cotton and soybean crops. Fungal strands and root necrosis were observed on roots two weeks before above ground plant symptoms were visible. Similarly, the system has provided information on the interactive effect of salinity and *Phytophthora parasitca* Dastur root rot, on root growth and senescence in tomato (*Lycopersicon esculentum* Mill., Snapp and Shennan 1994). The system also enabled a greater and earlier root senescence to be directly observed in plants subjected to both salt stress and fungal inoculation (Snapp and Shennan 1992, 1994). Similarly minirhizotron studies showed root colonisation by an arbuscular mycorrhizal fungus (*Glomus mossae* (Nic.& Gerd.) Gerdemann & Trappe) reduced root longevity of poplar (*Populus generosa inter americana* Beupre) roots (Hooker *et al.* 1995) as after 21 days, five times as many colonised as non-colonised poplar roots had died.

A preliminary experimental trial was therefore undertaken to ascertain if the minirhizotron-borescope system could be used to measure the affect of pasture root pathogens *Cylindrocladium scoparium* and *Fusarium crookwellense* on growth and development of white clover and perennial ryegrass roots.

3.2.5.2 Materials and Methods

Twelve plastic containers (30 cm deep x 25 cm wide) were placed on watering trays, and a clear plexiacrylic minirhizotron tube (50 cm in length, 2.5 cm in width) was inserted in each container at a 45° angle, so that half the minirhizotron tube was inside the container to a depth of 25 cm. Each container was then filled with Horotui soil previously sieved to 2 mm particles and the soil then compacted around the minirhizotrons. Before the minirhizotrons were covered in soil, three transect lines were marked with permanent coloured markers. The transect lines were drawn vertically for 25 cm down the length of each minirhizotron, with a different colour (red, yellow, green) so that a line was drawn on the top, right and left side of each tube. Horizontal lines (2.5 cm apart) were then drawn across the minirhizotrons to link each transect line creating 10 sections on each tube.

Twenty day old plants of perennial ryegrass (endophyte-free Nui) and white clover (Huia) germinated from seed were transplanted into the soil so that six of the containers were each planted with three clover plants and six each with three ryegrass plants. These plants were left to grow for a further 20 days before being inoculated with conidial suspensions of *C. scoparium* and *F. crookwellense*. Plants in each container were inoculated with 500 ml of conidial suspensions (10^8 conidia/ml), produced by growing each fungus on PCA at 25°C for 3 weeks. The suspension was pipetted into the soil of each container up to a depth of 10 cm (measured with a marked pipette) and all containers were inoculated three times, 3 days apart, to ensure dissemination of conidia through the soil. Four containers (two clover and two ryegrass) were inoculated with sterilised conidial suspensions to act as controls. Plants were maintained in a greenhouse at 16-20°C for the duration of the trial. Each treatment was replicated twice.

Twenty days after transplanting, the first root count measurements were undertaken. A root viewing borescope was used to count, at 7 day intervals, the number of roots observable of roots observable at the minirhizotron tube-soil interface. The borescope was moved down the all three vertical transect lines and the total number of intersecting roots were counted for each 2.5 cm section length in each tube. Root counts were made in eight out of the ten sections, the first and last 2.5 cm sections being excluded from the counts to avoid so called edge effects. Root measurement was continued for 12 weeks after the initial reading. Five weeks after transplanting the plants and soil were inoculated with fungal suspensions, roots were then measured twice weekly for four weeks after this event

to observe any rapid changes in root growth, after which counts were again undertaken at weekly intervals. At each observation date all roots counted were also mapped by drawing each visible root onto a transect data map (Appendix 8), to monitor root extension (root length mm) and root senescence in the same eight sections along each transect line. Different coloured pens were used to trace new root growth (all roots previously not drawn) as well as root death (disappearance or browning of roots drawn earlier). The length associated with each colour on a root map was calculated to obtain the accumulated totals of root growth and death over time, and the net root growth.

At the completion of the trial, surface-sterilised 3 mm root segments (20 from each plant) were plated onto WA to reisolate the inoculated fungi, and the remaining root and shoot components were dried in an oven for 48 hr at 60°C to determine dry weight yields. All data were analysed by analysis of variance using LSD tests for mean separation.

RESULTS

3.3.1 PATHOGENICITY OF ROOT FUNGI TO AXENICALLY GROWN SEEDLINGS.

3.3.1. (a) *In vitro* pathogenicity Petri plate test.

A rapid *in vitro* Petri plate technique was used to assess potential pathogenicity of fungi isolated from roots (Waipara *et al.* 1996a, Appendix 9).

Within 10 days disease symptoms had appeared on susceptible host seedlings and is allowed a pathogenicity rating to be calculated. A larger number of leguminous seedlings exhibited disease symptoms from a wide range of fungi than grass seedlings (Figure 3.3). Red clover was the most susceptible host with 33 species of fungi causing disease, while soft brome and tall fescue were susceptible to only nine and seven species respectively. Lotus was the least susceptible legume showing susceptibility to 18 species which was less than the most susceptible grasses (perennial ryegrass, Yorkshire fog and cocksfoot).

Declining productivity and poor persistence of forage legumes in pastures has been widely reported (Leath 1989) and these results further demonstrate that there are many species of root-invading fungi with potential pathogenicity to pasture plants.

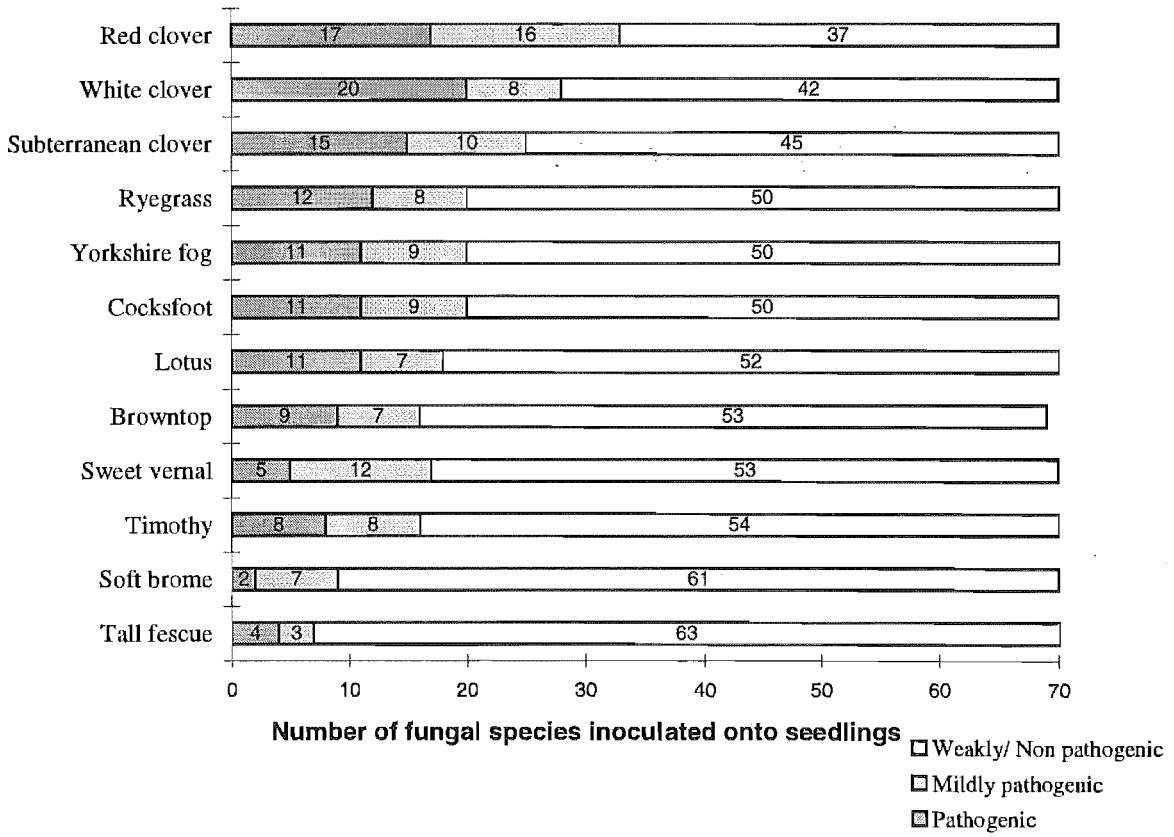


Figure 3.3 Number of pathogenic, mildly pathogenic and non-pathogenic fungi to twelve pasture species.

Of the 70 species of fungi inoculated onto seedlings (Table 3.7), 25 species were classed as pathogenic or mildly pathogenic to at least one host. However, hosts varied in susceptibility to individual isolates. Nine species of *Fusarium* showed a broad host range and were virulent pathogens with high disease scores of 4-5. Other pathogenic species with broad host ranges were *Cylindrocladium scoparium, Rhizoctonia solani, Botrytis cinerea* and *Phymatotrichum omnivorum* (Table 3.7). The greater susceptibility of leguminous seedlings was further demonstrated as isolates of *Cylindrocarpon destructans, Myrothecium verrucaria* and *Plectosporium tabacinum* were pathogenic to legumes but only classed as mildly, weakly, or non-pathogenic to grasses. *Preussia aemulans* was a host-specific pathogen, as it was pathogenic only to red clover and non-pathogenic to all of the other hosts tested (Table 3.7). Despite the greater susceptibility of clover seedlings, grass seedlings were susceptible to root damage by many of the fungi tested.

Table 3.7 Fungi pathogenic to seedlings of one or more pasture legume or grass species using the Petri plate screening test.

Fungal species	PATHOGENICITY SCORE											
	RC*	**WC**	**SC**	**RG**	**YF**	**CF**	**LO**	**SV**	**BT**	**TI**	**SB**	**TF**
Acremonium strictum	3**	3	3	0	1	3	0	2	2	2	0	1
Botrytis cinerea	3	3	2	3	3	3	2	2	3	2	1	1
Clasterosporium sp.	3	3	2	3	0	3	0	1	1	0	0	1
Codinaea fertilis	2	2	0	1	1	0	1	1	2	3	0	0
Colletotrichum sp.	3	3	2	1	1	1	2	0	2	0	0	0
Cylindrocarpon destructans	3	3	3	3	1	0	0	0	0	0	0	0
Cylindrocladium scoparium	3	3	3	3	3	3	3	2	3	2	2	2
Dreschlera dematioidea	2	2	2	2	2	2	1	1	2	2	2	1
Fusarium acuminatum	3	3	3	3	3	3	3	2	3	3	3	3
F. avenaceum	3	3	3	3	3	3	3	3	3	3	3	3
F. crookwellense	3	3	3	3	3	3	3	3	3	3	2	3
F. culmorum	3	3	3	3	3	3	3	2	3	3	1	3
F. equiseti	3	3	3	1	2	2	2	2	1	1	2	1
F. oxysporum	3	3	3	1	3	3	3	2	3	3	0	0
F. sambucinum	1	3	3	3	3	2	3	1	2	2	2	1
F. solani	2	3	3	2	2	2	3	1	1	1	0	1
F. tricinctum	3	3	3	3	3	3	3	3	3	3	1	3
Gliocladium roseum	2	2	3	1	3	2	2	2	1	1	1	1
Idriella bolleyi	2	3	3	3	2	2	2	3	1	0	0	0
Mortierella gamsii	3	3	3	1	1	0	2	1	1	1	2	0
Myrothecium verrucaria	3	3	3	2	2	2	1	1	1	2	0	1
Phymatotrichum omnivorum	3	3	1	2	2	2	1	2	3	2	0	1
Plectosporium tabacinum	3	3	3	2	2	1	2	2	1	0	0	0
Preussia aemulans	3	0	0	0	0	0	0	0	0	0	0	0
Rhizoctonia solani	3	3	2	2	3	3	3	3	3	3	0	2

*** RC** red clover, **WC** white clover, **SC** subterranean clover, **RG** ryegrass, **YF** Yorkshire fog, **CF** cocksfoot, **LO** lotus, **SV** sweet vernal, **BT** browntop, **TI** timothy, **SB** soft brome, **TF** tall fescue.

**** 3** pathogenic (average disease scores 4-5), **2** mildly pathogenic (average disease scores 3 -3.9), **1** weakly pathogenic (average disease scores 2- 2.9), **0** non-pathogenic (average disease scores 0 - 1.9).

Pathogenic fungi caused conspicuous symptoms on roots of susceptible hosts (Figures 3.4, 3.5, 3.6) including superficial brown to black discoloured streaks or patches, particularly at the root tips which caused root elongation to cease. These discoloured patches often coalesced to form discrete lesions or spread along infected roots until systemic root necrosis occurred (Figure 3.6). Systemic root necrosis usually caused legume

cotyledons, stems, and grass tillers to wilt and become chlorotic. Pathogenic fungi such as *F. tricinctum* colonised both root and shoot tissues and caused rapid seedling death (Figures 3.4, 3.5) .

Stunting or root tip atrophy was observed in some seedlings infected by moderately pathogenic fungi (Figure 3.7). These fungi did not cause seedling death but rather inhibited or stunted root development. Small superficial lesions and flecks of brown discoloration were also common symptoms induced by these moderately pathogenic fungi. *Arthrinium arundinis, Fusarium graminum, Thielaviopsis basicola* and *Trichoderma* spp. were all mildly pathogenic to many of the hosts tested. *Trichoderma* spp. produced variable disease symptoms on the seedlings, as disease scores on the same plate often ranged from 0-5, which may indicate this screen is not a reliable test of pathogenicity for this genus

The majority of fungi tested were either weakly pathogenic or non-pathogenic (Table 3.8, Figure 3.3) to all host seedlings. Weak pathogens induced discoloration of seedling roots or slight lesions (Figure 3.8), symptoms which were never observed on control seedlings. Sixteen fungi including all *Penicillium* spp. were classed as non-pathogenic as they induced no disease symptoms on any hosts (Table 3.8). Most of the remaining fungi were also classed as non-pathogenic to most hosts, but did induce disease symptoms in a few of the hosts. For example *Bimuria novae zelandiae* was mildly pathogenic to only red clover and *Mortierella alpina* was weakly pathogenic to only soft brome. *Curvularia trifolii* was weakly or moderately pathogenic to only the leguminous hosts, a further example of these plants being more susceptible to disease than grasses.

Tall fescue and soft brome were noticeable by their average disease scores of 0 for most hosts. Both hosts, but in particular soft brome, had numerous root hairs on seedling roots which may have acted as a physical barrier to decrease or inhibit fungal colonisation for the first ten days when pathogenicity was assessed.

Fungi considered to be non-pathogenic produced no disease symptoms on infected seedlings (Figure 3.9) which resembled control seedlings and all had white, turgid roots in which there was no inhibition of growth.

Table 3.8 Species of fungi classed as weakly or non-pathogenic to pasture legume and grass seedlings using a Petri plate technique.

Fungal species	PATHOGENICITY											
	RC*	**WC**	**SC**	**RG**	**YF**	**CF**	**LO**	**SV**	**BT**	**TI**	**SB**	**TF**
Acremonium curvulum	1	1	1	0	0	0	0	1	1	0	0	0
Acremoniella atra	0	0	1	1	1	0	0	0	0	0	0	0
Arthrinium arundinis	1	2	1	0	1	1	0	1	1	2	0	0
Aspergillus niger	0	0	0	0	0	0	0	0	0	0	0	0
Aspergillus ustus	0	0	0	1	0	0	1	0	0	0	0	0
Bimuria novae zelandiae	2	0	0	0	0	0	0	0	0	0	0	0
Chaetomium funicola	0	0	1	1	0	0	0	0	0	0	0	0
Chaetomium globosum	0	0	0	0	0	0	0	0	0	0	0	0
Curvularia trifolii	2	2	1	0	0	0	1	0	0	0	0	0
Dactylaria acerosa	0	0	0	0	0	0	0	0	0	0	0	0
Fusarium graminum	2	2	2	0	2	1	1	0	0	1	0	0
Gongronella butleri	0	0	0	0	0	0	0	0	0	0	0	0
Mariannaea elegans	0	0	0	0	0	0	0	0	0	0	0	0
Metarhizium anisopliae	0	0	0	0	0	0	0	0	0	0	0	0
Mortierella alpina	0	0	0	0	0	0	0	0	0	0	1	0
M. elongata	0	0	0	0	0	0	0	0	0	0	0	0
M. globulifera	0	0	1	1	0	0	0	0	0	0	0	0
Paecilomyces carneus	0	0	0	0	0	0	0	0	0	0	0	0
Paecilomyces lilacinus	0	1	1	1	0	0	1	0	1	1	1	0
Paecilomyces marquandii	0	0	0	0	0	0	0	0	0	0	0	0
Pithomyces chartarum	0	0	0	2	0	0	0	0	NT	0	0	0
Periconia macrospinosa	2	0	1	1	0	0	0	0	1	0	0	0
Penicillium brevicompactum	0	0	1	0	0	0	1	0	0	0	0	0
P. chrysogenum	0	0	0	0	0	0	0	0	0	0	0	0
P. decumbens	0	0	0	0	0	0	0	0	0	0	0	0
P. griseofulvum	0	0	0	0	0	0	0	0	0	0	0	0
P. janczewskii	0	0	0	0	0	0	0	1	0	0	0	0
P. janthinellum	0	0	0	0	1	0	1	0	0	0	0	0
P. oxalicum	0	0	1	0	0	0	0	0	0	0	0	0
P. simplicissimum	0	0	0	1	0	0	0	0	0	0	1	0
P. variable	0	0	1	0	0	0	0	0	0	0	0	0
Ramichloridium schultzeri	1	0	0	1	0	0	1	2	0	0	0	0
Sordaria fimicola	0	0	0	1	0	0	1	0	1	0	0	0
Tetraploa aristata	0	2	0	0	0	0	0	0	0	0	0	0
Thielaviopsis basicola	2	1	1	0	0	1	1	2	1	1	0	1
Thozetella tocklaiensis	0	0	0	0	0	0	0	0	0	0	0	0
Tricellula aquatica	0	0	0	0	0	0	0	0	0	0	0	0
Trichoderma hamatum	1	2	1	2	0	1	0	0	1	1	1	0
T. harzianum	2	1	2	1	0	2	2	1	1	1	2	1
T. koningii	1	0	2	1	1	1	3	0	0	0	1	0
T. polysporum	2	0	1	3	0	0	1	0	0	0	2	1
T. viride	2	0	1	1	0	0	0	1	1	0	1	1
Trichosporon cutaneum	0	0	0	0	0	0	0	0	0	0	0	0
Verticicladiella sp.	0	0	0	0	0	0	0	0	0	0	0	0
Verticillium chlamydosporium	0	0	0	0	0	0	0	0	0	0	0	0

*** RC** red clover, **WC** white clover, **SC** subterranean clover, **RG** ryegrass, **YF** Yorkshire fog, **CF** cocksfoot, **LO** lotus, **SV** sweet vernal, **BT** browntop, **TI** timothy, **SB** soft brome, **TF** tall fescue.

** **3** pathogenic (average disease scores 4-5), **2** mildly pathogenic (average disease scores 3 -3.9), **1** weakly pathogenic (average disease scores 2- 2.9), **0** non-pathogenic (average disease scores 0 - 1.9).

NT = Not tested due to contamination.

Inoculated fungi were readily reisolated from root segments plated onto WA at the completion of the experiment. Contamination of the seedling plates by other fungi was rare.

This *in vitro* test provided a preliminary indication of potential root colonisation, host specificity and pathogenicity of fungi to host seedlings. However, when seedlings are grown in axenic culture on an agar medium the incoculated fungus will be favoured rather than the plant host. Results may not indicate what would happen under field conditions which are affected by microbial interactions in the rhizosphere and by other environmental factors.

Figure 3.4 Seedling mortality of browntop inoculated with *Fusarium tricinctum*

Figure 3.5 Seedling mortality of subterranean clover inoculated with *Fusarium tricinctum*

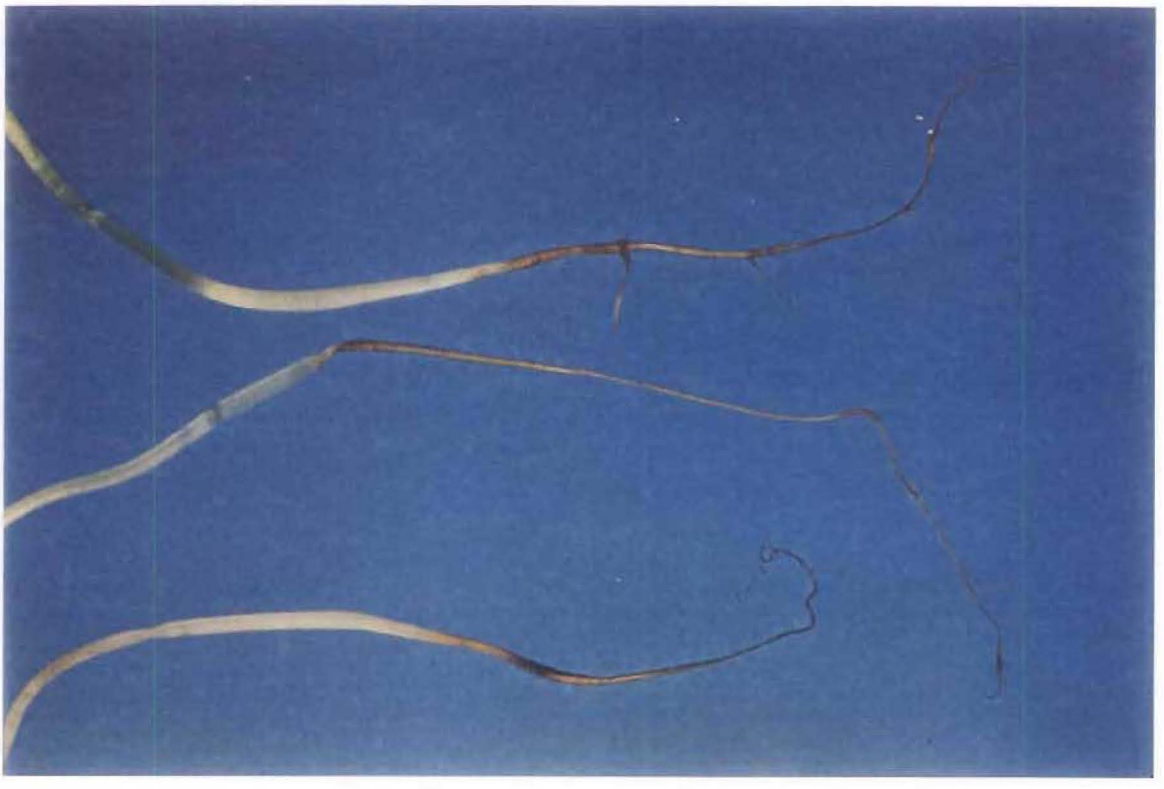


Figure 3.6 Typical disease symptoms on seedling roots inoculated with pathogenic fungi.

Figure 3.7 Root tip atrophy of white clover seedlings inoculated with *Tetraploa aristata*.

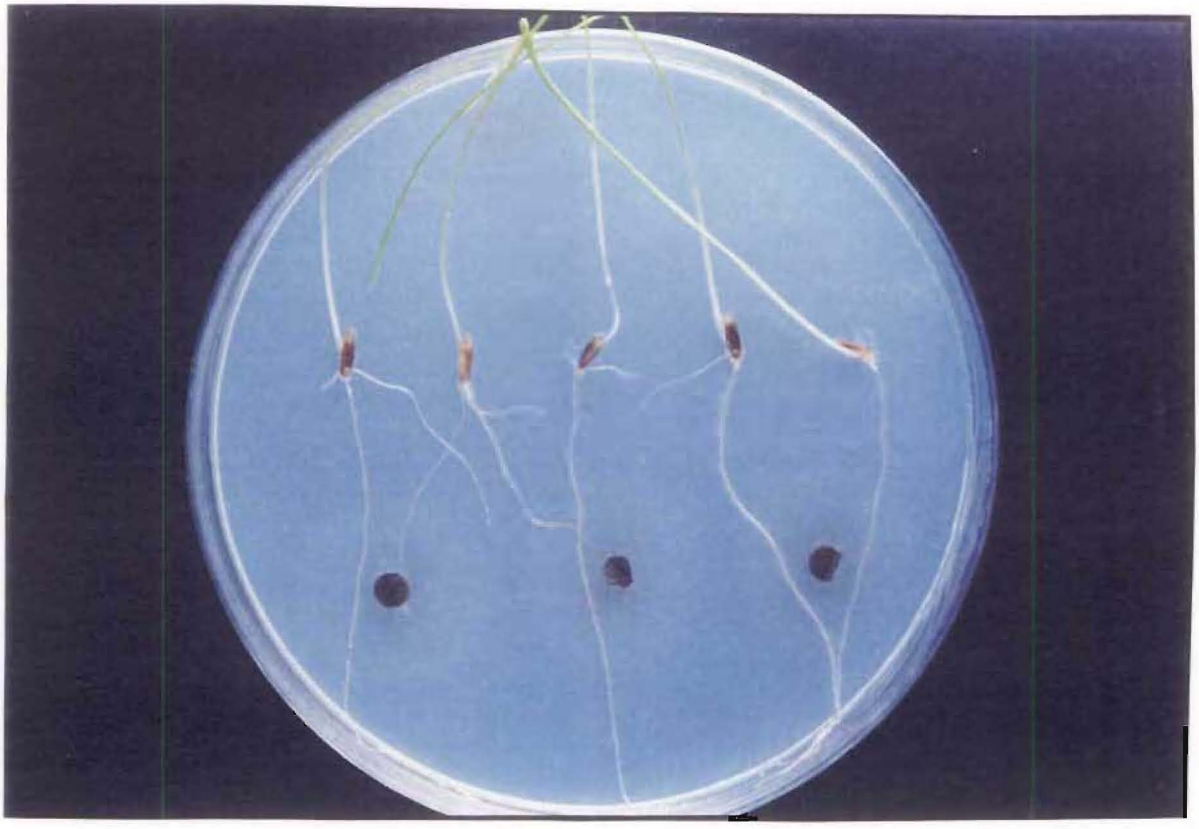


Figure 3.8 Light brown discoloration or 'bronzing' of perennial ryegrass seedlings inoculated with *Acremoniella atra*.

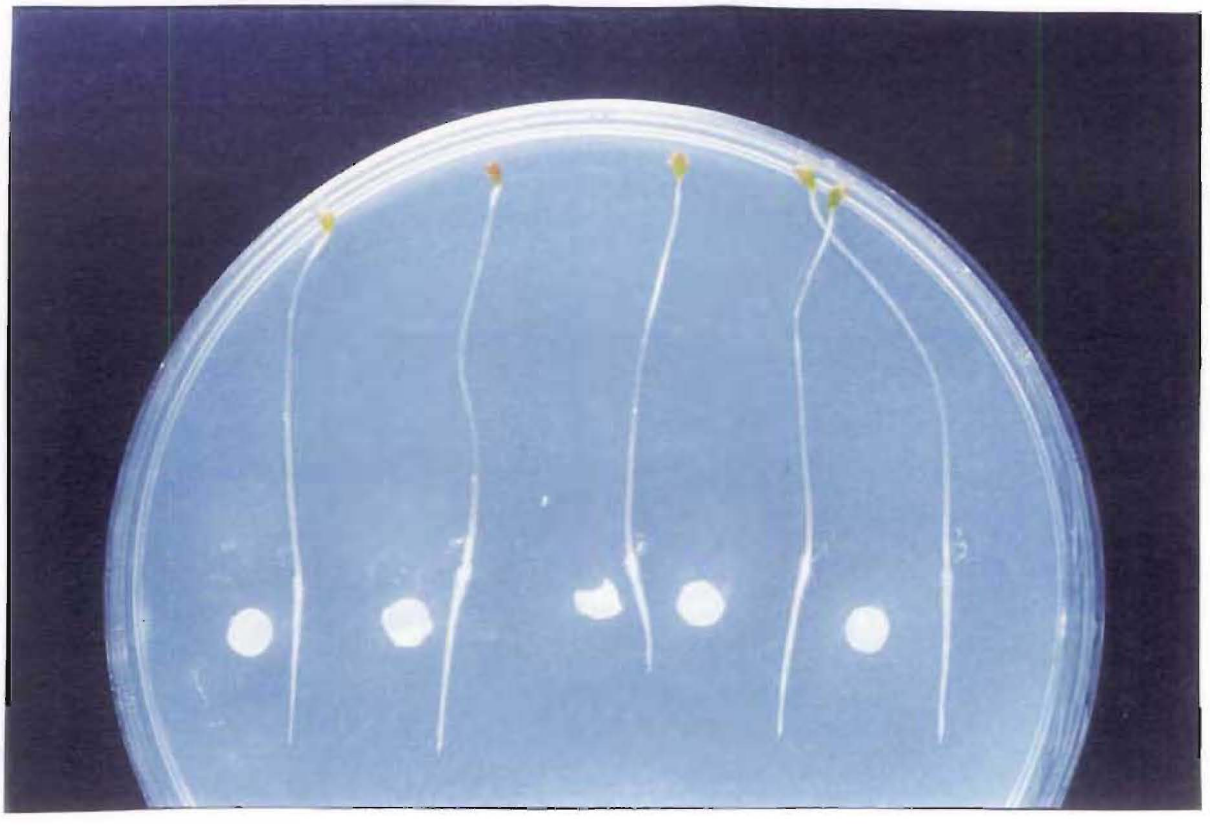


Figure 3.9 White clover seedlings showing no disease symptoms after being inoculated with *Trichosporon cutaneum*.

3.3.1 (b) Staining method to assess fungal colonisation.

All species of fungi inoculated onto seedlings, were observed to have penetrated the stained root sections of at least one host (Table 3.9), confirming them as root-invading fungi and satisfying Koch's postulates. Hyphae of pathogens, such as *C. scoparium* and *Fusarium* spp., systemically colonised the vascular and inner cortex of the hosts (Figure 3.10). Most fungal hyphae, however, were only observed in the cortical or epidermal tissues and so were rated with 0 and 1 colonisation scores. *Trichoderma viride* was observed once in the epidermis of white clover showing it to have the weakest root-invading capacity of all the fungi tested.

Table 3.9 Fungal colonisation of inoculated seedlings of twelve pasture plants.

Fungal species	COLONISATION SCORES											
	RC*	WC	SC	RG	YF	CF	LO	SV	BT	TI	SB	TF
Acremoniella atra	2	1	2	1	1	1	1	1	1	1	1	1
Acremonium curvulum	2	2	1	1	1	1	1	2	2	2	0	1
Acremonium strictum	2	2	1	1	2	1	1	1	3	1	0	1
Arthrinium arundinis	2	2	2	0	2	1	1	1	2	1	0	0
Aspergillus niger	1	1	1	1	1	1	1	1	1	1	0	1
Aspergillus ustus	1	1	1	1	1	1	1	1	0	1	1	1
Bimuria novae zelandiae	2	1	1	0	0	0	1	0	0	0	0	0
Botrytis cinerea	2	2	2	1	3	1	2	2	3	0	1	0
Chaetomium funicola	0	1	1	1	0	1	1	0	0	1	1	1
Chaetomium globosum	1	1	1	0	1	1	1	1	NT	1	0	1
Clasterosporium	2	2	1	1	1	1	1	1	1	1	1	1
Codinaea fertilis	2	2	2	1	1	2	1	1	3	2	0	1
Colletotrichum sp.	2	2	2	2	1	1	1	2	1	0	0	0
Curvularia trifolii	2	2	2	2	1	1	1	1	1	1	1	1
Cylindrocarpon destructans	2	2	2	2	2	1	1	1	3	1	1	2
Cylindrocladium scoparium	3	3	3	3	3	3	3	3	3	2	2	2
Dactylaria acerosa	1	1	1	1	1	0	1	1	1	1	0	1
Dreschlera dematioidea	3	1	2	1	3	2	1	3	3	3	2	2
Fusarium acuminatum	2	3	3	2	1	2	2	3	2	3	1	3
Fusarium avenaceum	3	2	3	3	3	3	2	3	3	3	1	3
Fusarium crookwellense	3	2	3	3	3	3	3	3	3	3	2	2
Fusarium culmorum	2	3	3	3	3	3	2	3	3	3	1	3
Fusarium equiseti	2	1	1	1	1	1	1	1	1	1	1	1
Fusarium graminum	1	1	3	2	2	2	2	1	2	2	0	0
Fusarium oxysporum	2	2	2	2	3	3	2	3	3	3	1	2
Fusarium sambucinum	1	1	3	2	3	3	2	2	3	3	1	1
Fusarium solani	2	1	2	2	1	1	2	2	3	2	1	2
Fusarium tricinctum	2	3	3	2	3	3	2	2	3	3	1	3
Gliocladium roseum	1	2	1	1	3	2	0	1	3	2	1	1
Gongronella butleri	1	1	1	1	1	1	1	1	0	1	1	1
Idriella bolleyi	1	1	2	1	1	2	2	1	3	0	1	1
Mariannaea elegans	1	1	1	1	1	1	1	1	1	1	1	1
Metarhizium anisopliae	0	1	0	0	0	1	0	1	1	0	0	0
Mortierella alpina	0	1	0	0	0	0	0	0	0	0	1	0
Mortierella elongata	1	1	0	0	1	0	1	1	0	0	1	1
Mortierella gamsii	1	1	1	1	1	1	1	1	1	0	1	1
Mortierella globulifera	1	1	1	1	1	1	1	1	0	0	0	1
Myrothecium verrucaria	1	1	1	1	0	1	1	1	3	0	0	0
Paecilomyces carneus	1	1	1	1	1	1	0	1	1	1	0	1
Paecilomyces lilacinus	1	1	1	1	1	1	1	1	1	1	0	1
Paecilomyces marquandii	1	1	1	1	1	1	1	1	1	1	0	1
Penicillium brevicompactum	1	1	1	1	1	1	1	1	1	1	0	1
P. chrysogenum	1	1	1	1	1	1	1	1	1	1	1	1

FUNGAL SPECIES	RC*	WC	SC	RG	YF	CF	LO	SV	BT	TI	SB	TF
P. decumbens	1	1	1	0	1	1	1	1	0	1	0	1
P. griseofulvum	0	1	1	0	1	1	0	1	1	1	0	1
P. janczewskii	1	1	1	1	1	1	1	1	1	1	1	1
P. janthinellum	1	1	1	1	1	1	1	1	1	1	1	2
P. oxalicum	1	1	1	1	1	1	1	1	0	1	0	0
P. simplicissimum	1	1	1	1	1	1	1	1	1	1	1	2
P. variabile	1	1	1	0	1	1	1	1	1	1	0	1
Periconia macrospinosa	3	2	3	1	2	1	2	2	3	3	1	2
Phymatotrichum omnivorum	3	3	1	2	1	2	2	1	3	1	1	2
Pithomyces chartarum	0	1	0	1	1	0	0	1	NT	0	0	1
Plectosporium tabacinum	2	2	2	2	1	1	1	1	3	1	1	2
Preussia aemulans	2	2	2	1	1	2	1	1	1	1	1	1
Ramichloridium schultzeri	1	1	1	1	1	1	1	1	1	1	1	1
Rhizoctonia solani	3	2	1	0	0	0	3	3	0	0	0	1
Sordaria fimicola	1	1	1	1	1	0	1	0	0	1	1	1
Tetraploa aristata	1	1	1	1	1	1	0	1	0	1	0	0
Thielaviopsis basicola	3	2	1	1	1	1	2	2	1	3	1	2
Thozetella tocklaiensis	1	1	1	1	2	1	1	1	0	1	0	1
Tricellula sp.	1	2	1	0	1	1	1	0	1	1	0	0
Trichoderma hamatum	1	1	1	0	1	1	1	1	1	1	0	1
Trichoderma harzianum	1	1	1	0	0	1	0	0	0	0	0	1
Trichoderma koningii	0	0	1	1	1	1	1	0	0	0	0	1
Trichoderma polysporum	1	1	0	1	1	1	1	1	1	0	0	0
Trichoderma viride	0	1	0	0	0	0	0	0	0	0	0	0
Trichosporon cutaneum	1	1	1	1	1	1	1	1	1	0	0	0
Verticicladiella sp.	1	1	1	1	1	1	1	1	1	1	1	1
Verticillium chlamydosporium	1	1	1	1	1	1	1	1	1	1	0	1

* **RC** red clover, **WC** white clover, **SC** subterranean clover, **RG** ryegrass, **YF** Yorkshire fog, **CF** cocksfoot, **LO** lotus, **SV** sweet vernal, **BT** browntop, **TI** timothy, **SB** soft brome, **TF** tall fescue. ** **3** = inner cortex and vascular tissue colonisation, **2** = cortex colonisation, **1** = epidermal colonisation, **0** = hyphal colonisation absent. NT = Not tested due to contamination.

The number of fungal species which colonised each host plant was variable (Figure 3.10). White clover was the most susceptible host with 69 fungi being observed in stained root sections, with only *Trichoderma koningii,* not being observed to have invaded any root tissues (Table 3.9). The other leguminous hosts also had high numbers (>60) of fungi able to colonise the axenically grown roots. In contrast seedlings of ryegrass, browntop, timothy, tall fescue and soft brome were found to have a low number of fungi able to colonise their roots (Table 3.10), for example only 37 fungi invaded the roots of soft brome. The number of fungi which colonised the remaining grasses Yorkshire fog, sweet vernal and cocksfoot, was similar that observed for leguminous hosts.

Table 3.10 Total number of fungi observed colonising stained seedling root sections.

Colonisation scores	Total number of fungi											
	RC*	WC	SC	RG	YF	CF	LO	SV	BT	TI	SB	TF
Zero colonisation (0 score)	7	1	6	14	7	8	9	8	16	18	34	14
Epidermal colonisation (1)	38	45	42	41	46	47	45	46	29	36	34	41
Cortical colonisation (2)	18	19	13	11	6	8	13	8	4	6	3	11
Vascular colonisation (3)	7	5	9	4	10	7	3	8	19	10	0	4
Total colonisation (1,2,3)	**63**	**69**	**64**	**56**	**63**	**62**	**61**	**62**	**52**	**52**	**37**	**56**

* **RC** red clover, **WC** white clover, **SC** subterranean clover, **RG** ryegrass, **YF** Yorkshire fog, **CF** cocksfoot, **LO** lotus, **SV** sweet vernal, **BT** browntop, **TI** timothy, **SB** soft brome, **TF** tall fescue.

Fungal colonisation of most seedlings was restricted to the epidermal tissues of all inoculated hosts (Figure 3.10) with fewer fungi being observed in either the cortex or vascular tissues. A greater number of fungi were observed to systemically penetrate clover roots rather than grass roots. Browntop roots had the most fungal species colonising vascular tissue, whereas soft brome had no vascular colonisation, and only 3 fungi invaded the cortex.

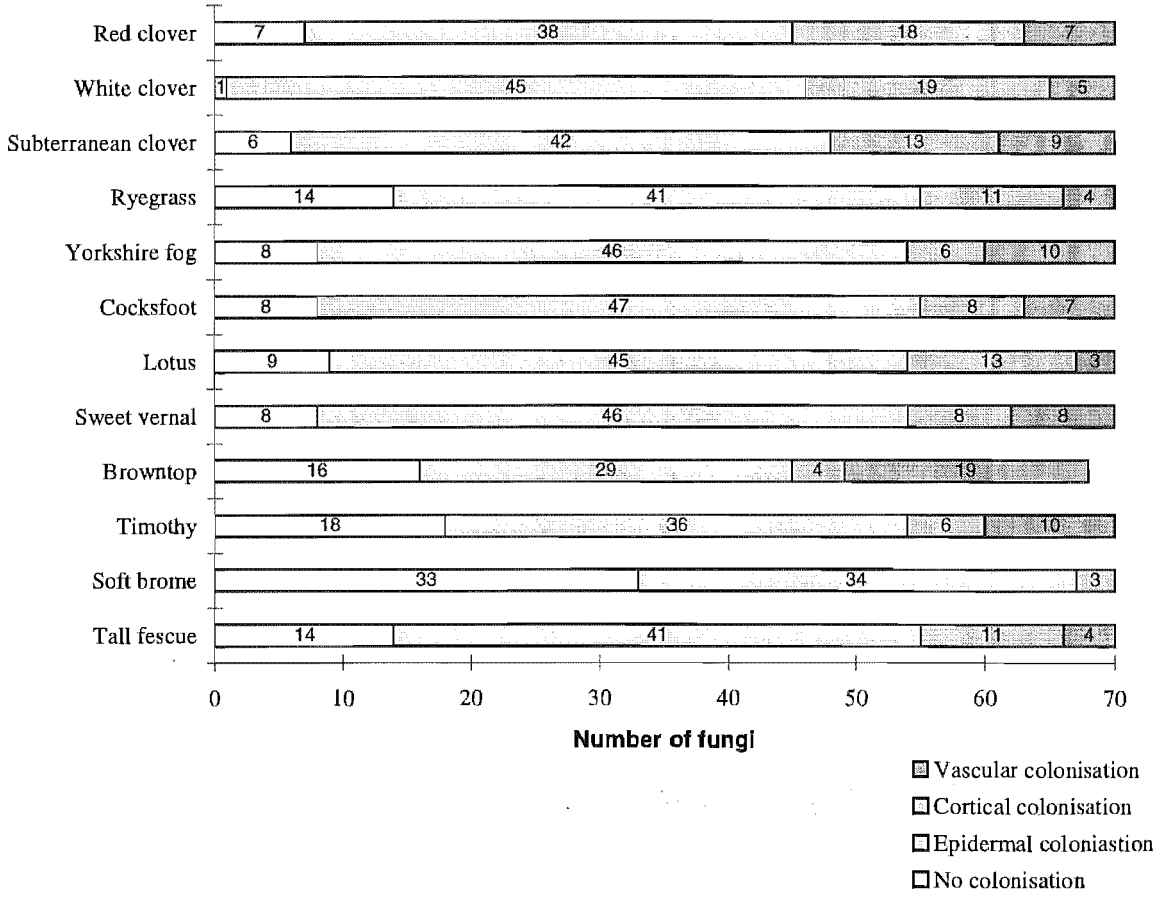


Figure 3.10 Number of fungi observed to have colonised the seedling root tissues of twelve pasture species.

Root-colonisation of inoculated seedlings was readily assessed by the clearing and staining of root sections. Both intercellular and intracellular fungal colonisation of the root, surface, epidermis, cortex and vascular cells were observed (Figures 3.11-3.12). Infection cushions or hyphal swellings were often associated with the fungal colonisation of infected root tissues (Figures 3.13, 3.14). Other fungal structures, such as the microsclerotia of *Cylindrocladium scoparium* (Figure 3.14) and *Phymatotrichum omnivorum*, and chlamydospores of *Periconia macrospinosa* were produced in infected root tissues. (Figures 3.15 - 3.16), *Fusarium acuminatum, Fusarium equiseti, Fusarium oxysporum, C. destructans* and *Thielaviopsis basicola* (Figure 3.17), were observed in some infected host tissues. Hulle cells of *Aspergillus ustus* were observed in the epidermal cells of white clover, subterranean clover, and lotus.

This Petri plate method has previously reported to induce sporulation in some fungi (Christensen *et al.* 1988), and many of the fungi tested here were observed to produce conidiophores and conidia at the agar-root interface. Hyphae in infected epidermal cells often developed numerous conidiophores at the root surface, thus triggering copious production of conidia (Figure 3.18). This technique also induced the production of sporodochia of fusaria (*Fusarium crookwellense, F. avenaceum, F. equiseti, F. culmorum* Figure 3.19), and *T. tocklaiensis,* and acervuli of *Colletotrichum,* were observed on inoculated roots. Sexual structures of ascomycetous species *Bimuria novae zelandiae* (Figure 3.20), *Preussia aemulans* (Figure 3.21) and *Sordaria fimicola* appeared at the agar-root interface. Structural characters not usually produced on standard media or incubation conditions were observed on inoculated roots. For example blastoconidia of *Phymatotrichum omnivorum* were observed on sweet vernal, tall fescue, cocksfoot, and ryegrass roots, while the rarely produced *Aspergillus*-like phialospore state of *Acremoniella atra* (Figures 3.22, 3.23), first reported by (Mason 1933), was seen on inoculated roots of red clover, subterranean clover and Yorkshire fog.

Symptomless colonisation of seedlings by non-pathogenic fungi as well as potentially pathogenic fungi was commonly observed underlining the importance of supplementing macroscopic examination of symptoms with microscopic examination of stained tissue to assess hyphal invasion. Symptomless infections have been referred to as latent, dormant, or quiescent (Bacon and Hinton 1996), and similar endophytic symptomless infections have been reported in several grasses (Bacon and Siegel 1988).

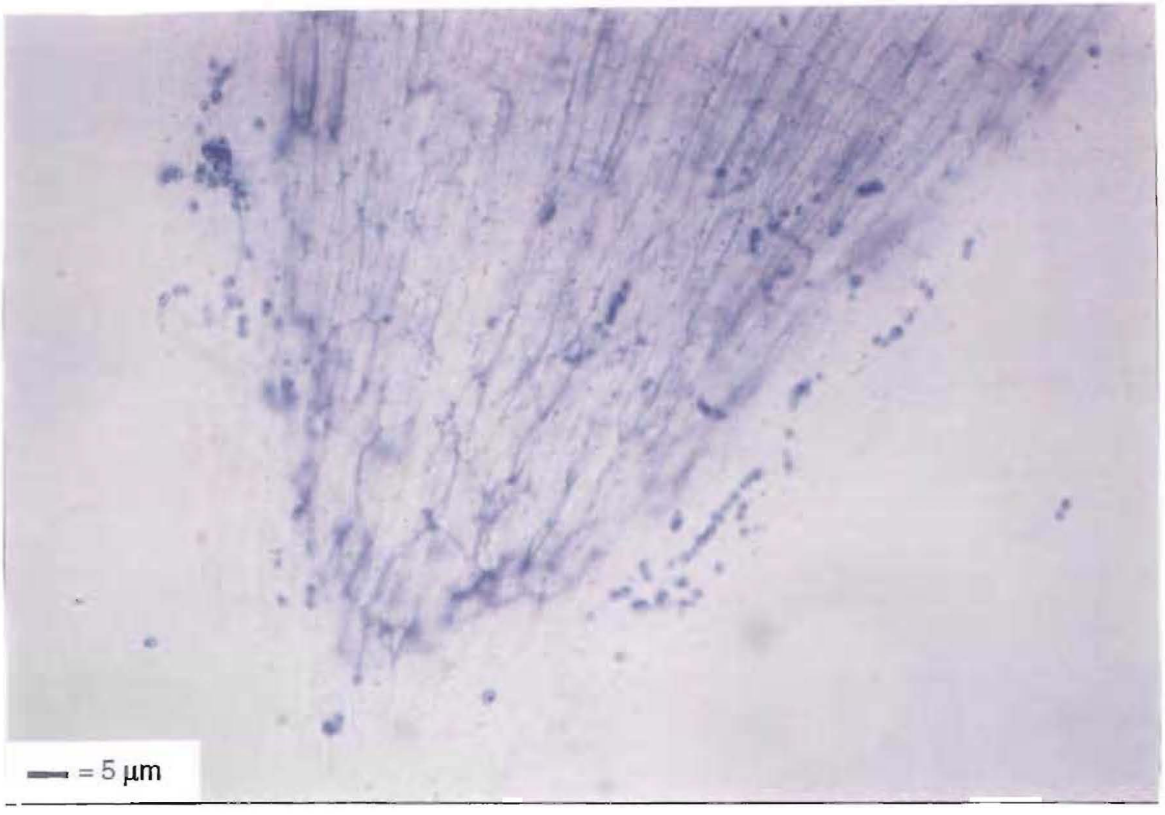


Figure 3.11 Cells of yeast *Trichosporon cutaneum* on an infected root tip of lotus.

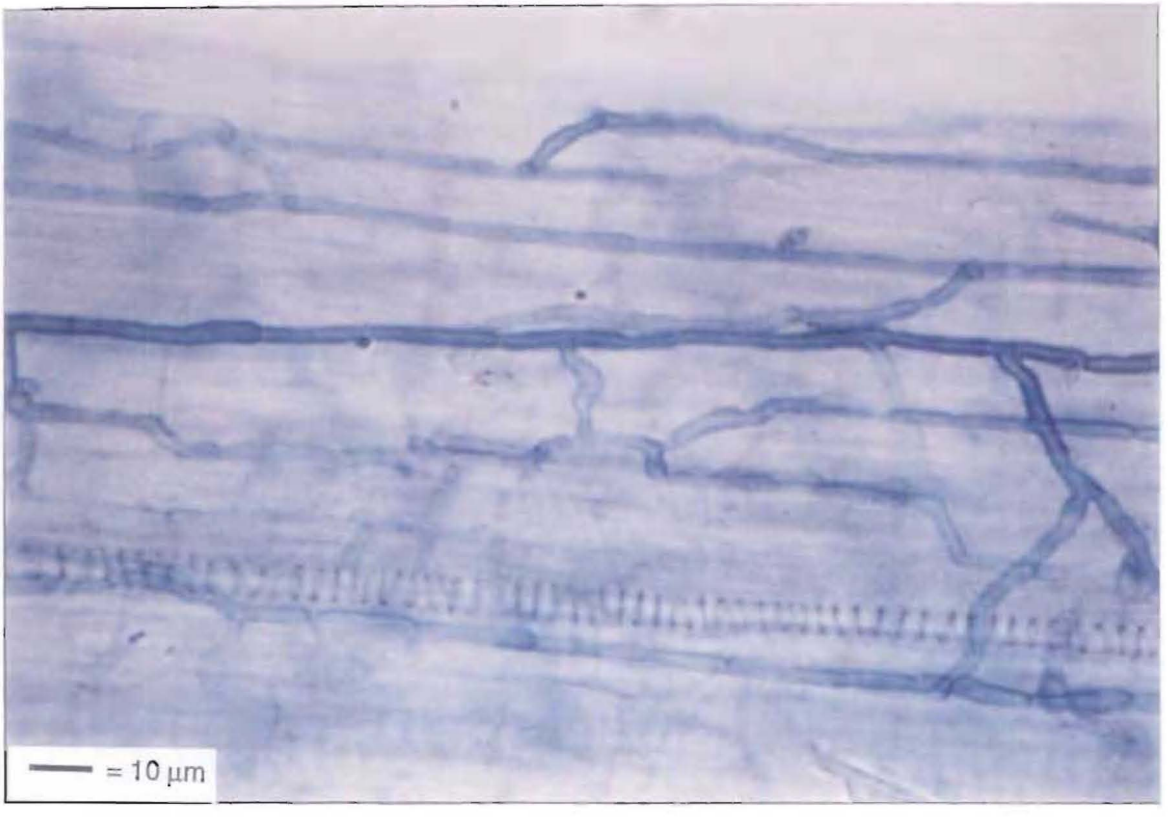


Figure 3.12 LS root of lotus, showing systemic intercellular hyphae of *Rhizoctonia solani.*

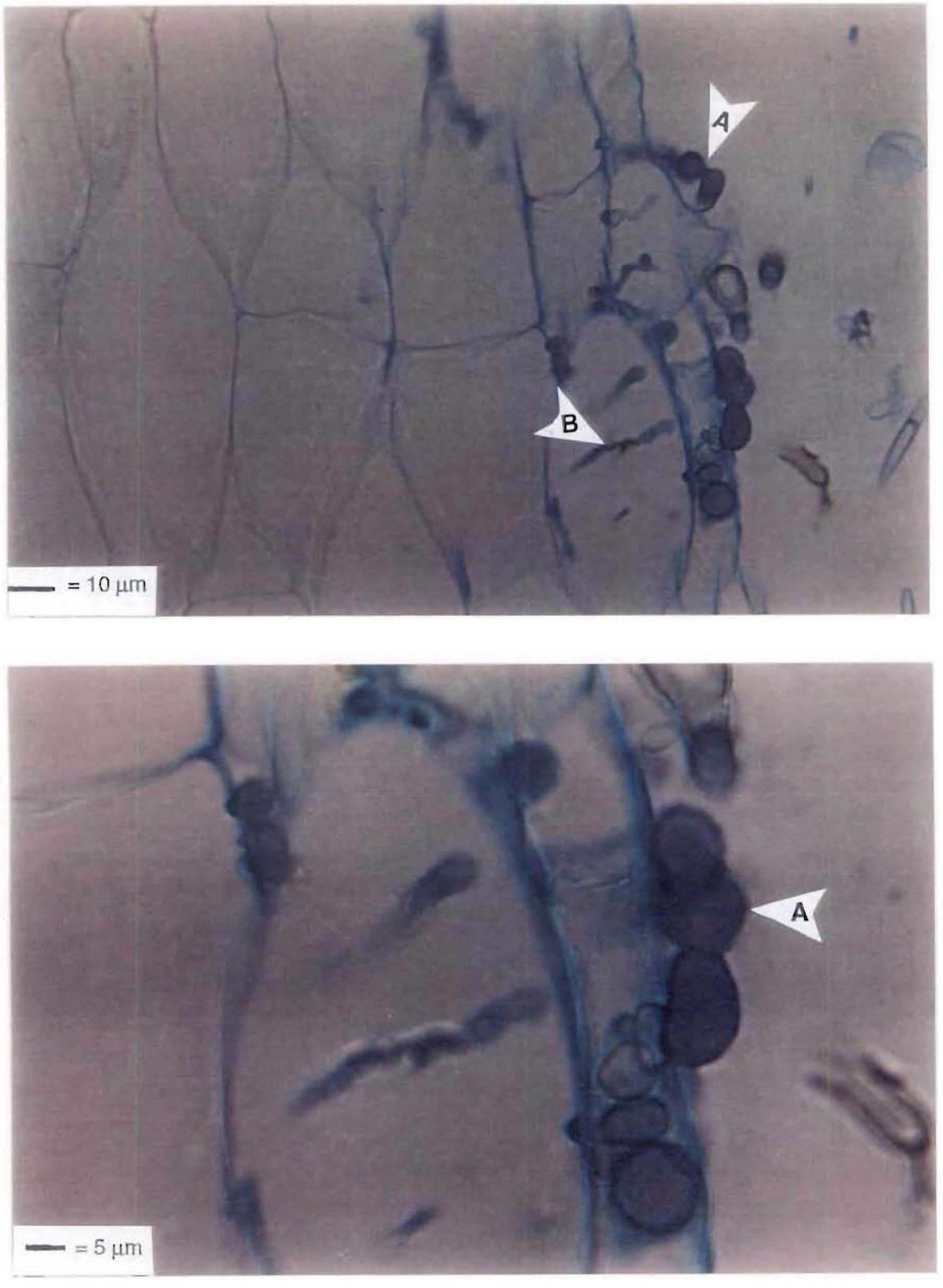


Figure 3.13 Infection cushions (A) of *Cylindrocarpon destructans* infecting the epidermis of red clover preceding the intracellular hyphal invasion of outer cortex cells (B). Top 400x magnification, Bottom 1000x magnification.

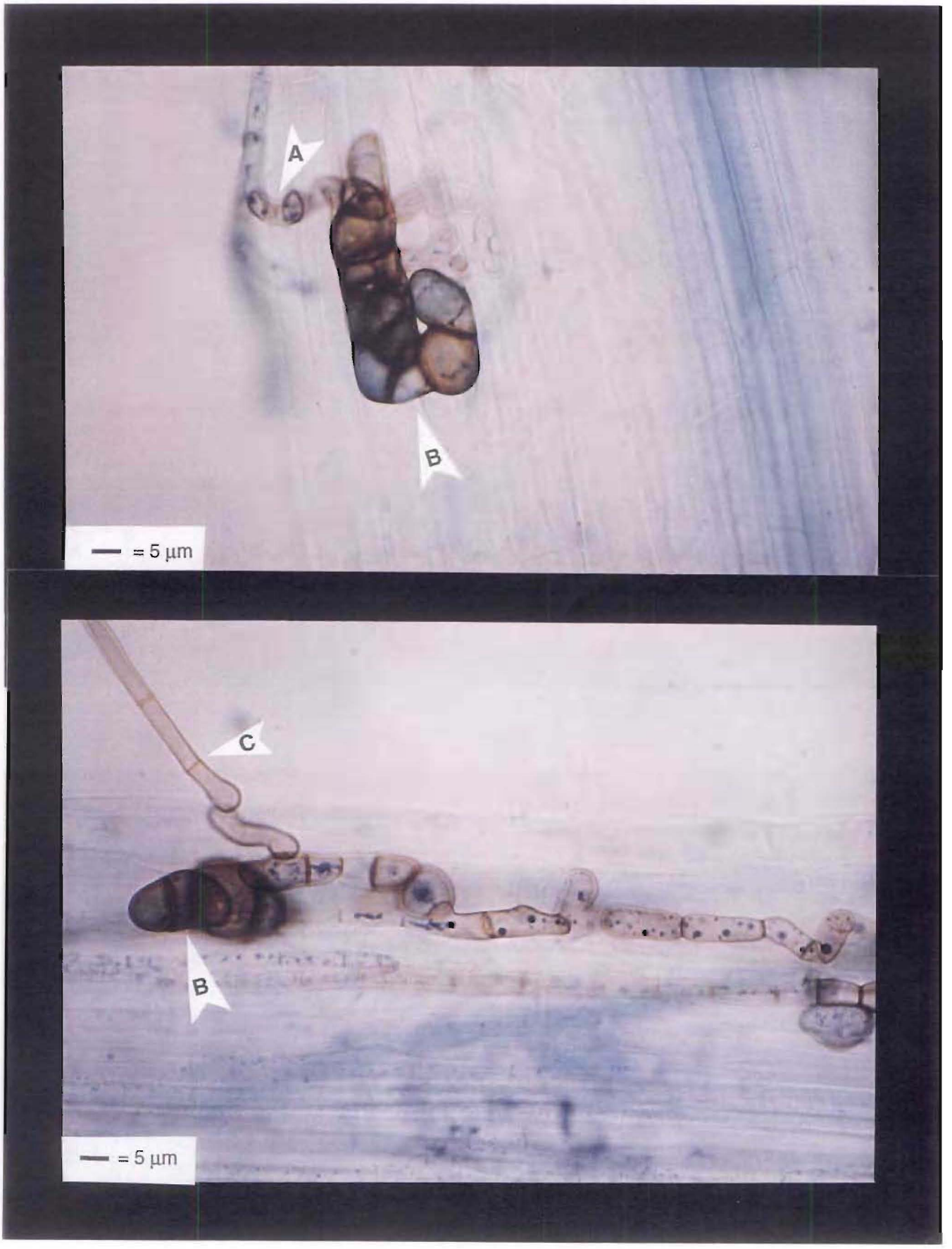


Figure 3.14 Intracellular infection cushions (A), intracellular microsclerotia (B), and conidiophore (C) of *Cylindrocladium scoparium* produced in the cortex of perennial ryegrass.

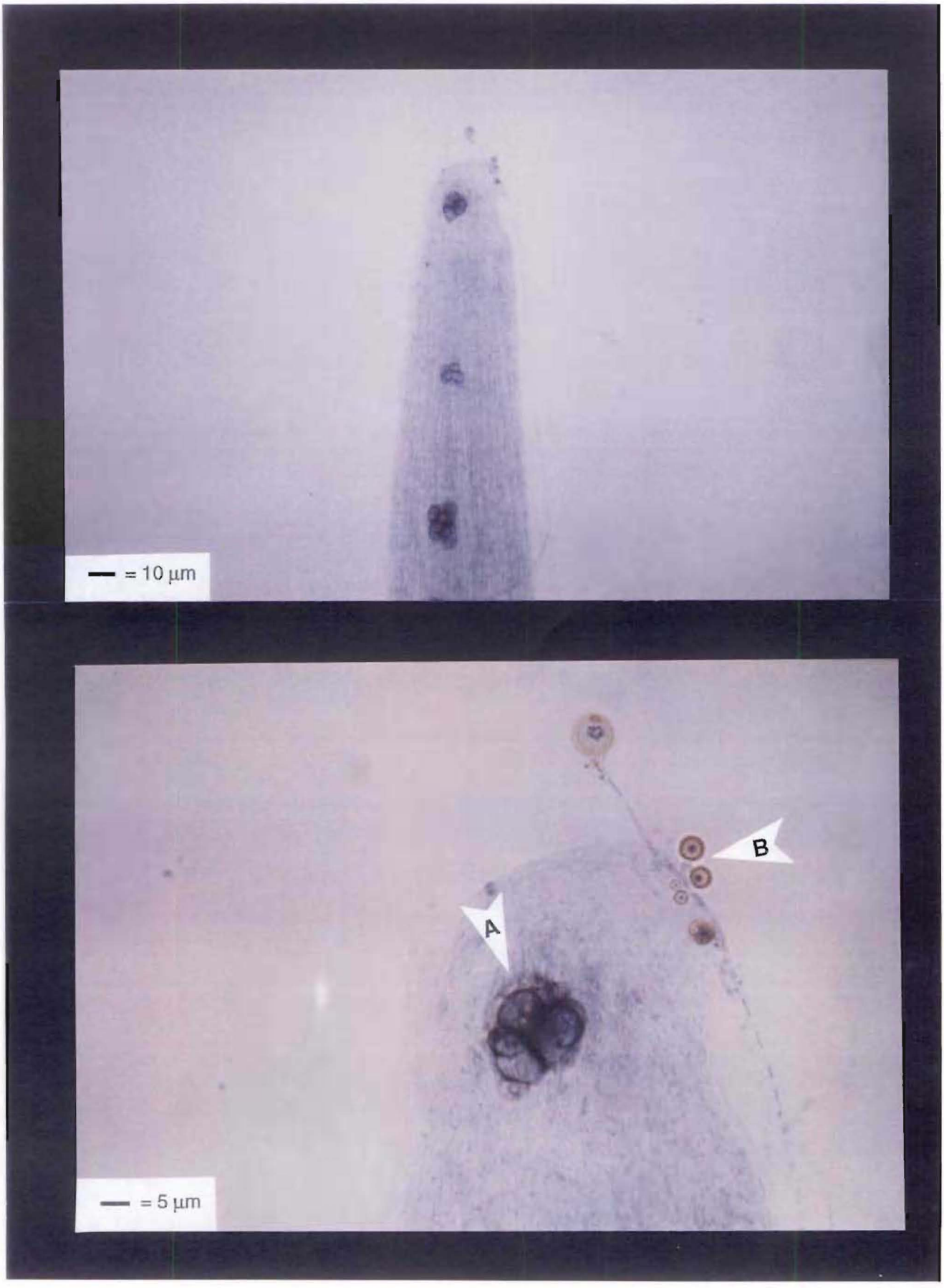


Figure 3.15 LS section of white clover roots showing the production of chlamydospores (A) and conidia (B) by *Periconia macrospinosa*. (Top 400x; Bottom 1000x)

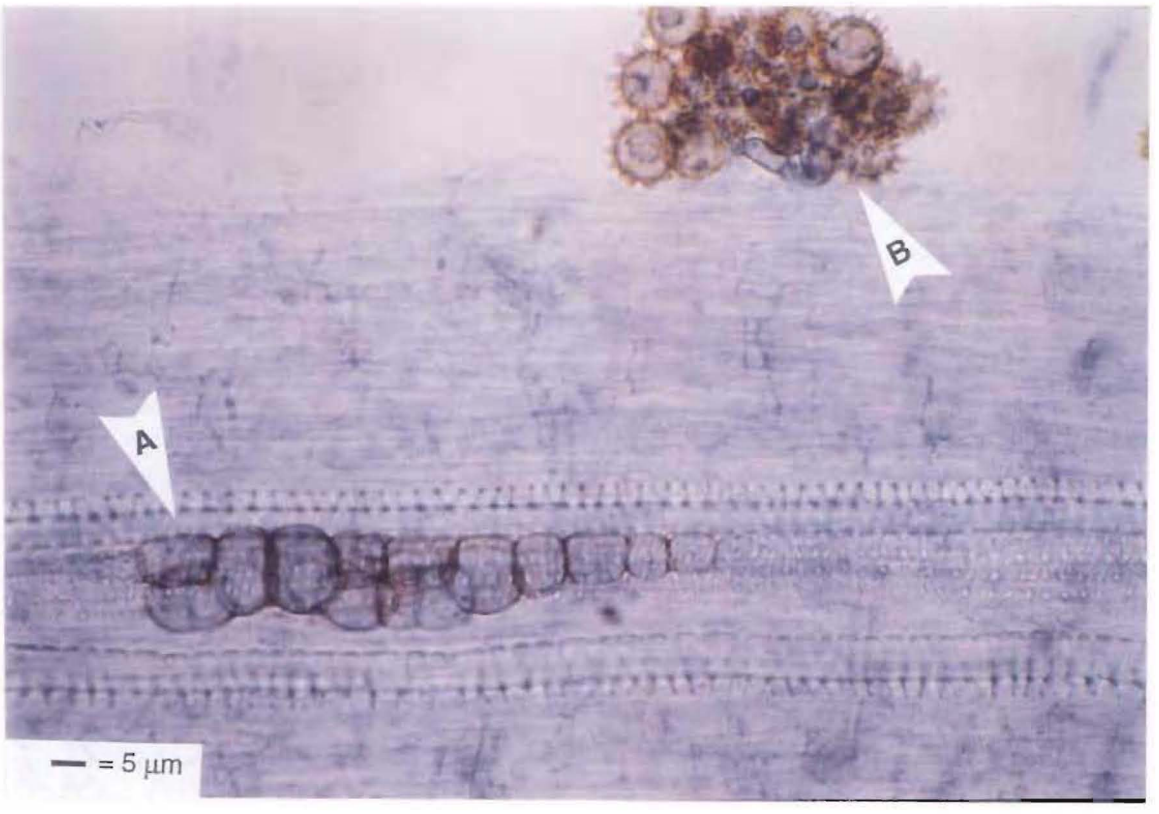


Figure 3.16 Production of chlamydospores (A) in the vascular tissues of perennial ryegrass infected by *Periconia macrospinosa,* conidia (B) are produced at the root surface.

Figure 3.17 Production of chlamydospores on the surface of a red clover root infected by *Thielaviopsis basicola* (400x).

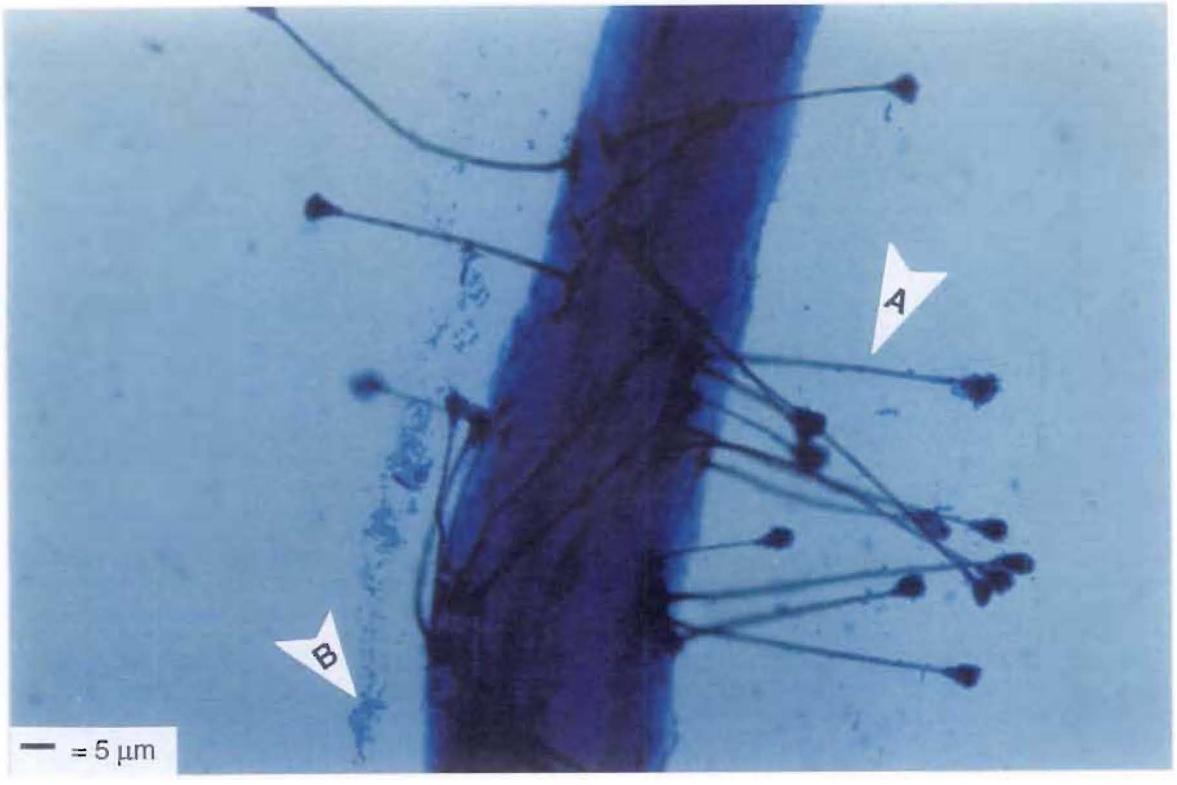


Figure 3.18 Production of conidiophores (A) and conidia (B), from a white clover root infected with *Verticicladiella* (400x).

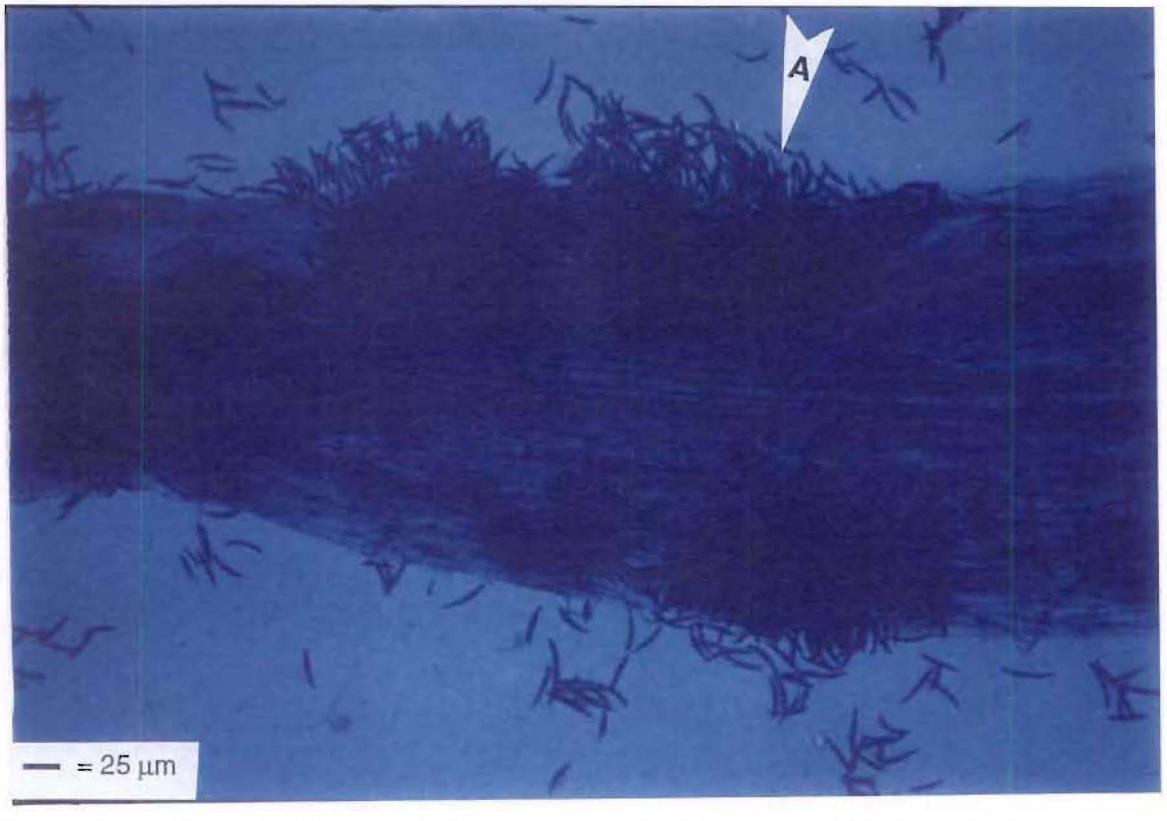


Figure 3.19 Sporodochia (A) of *Fusarium crookwellense* produced on the surface of an infected browntop root.

Figure 3.20 Ascomata of *Bimuria novae zelandiae* produced on the surface subterranean clover roots.

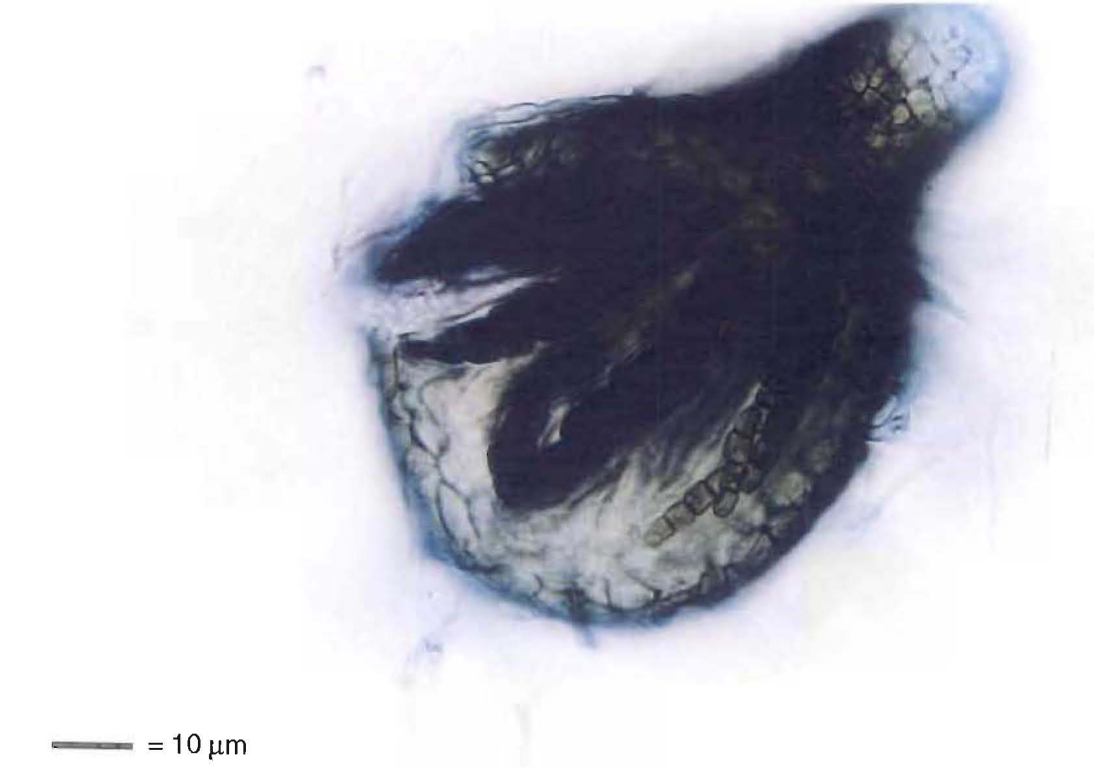


Figure 3.21 Ascomata of *Preussia aemulans* produced on the surface of cocksfoot roots.

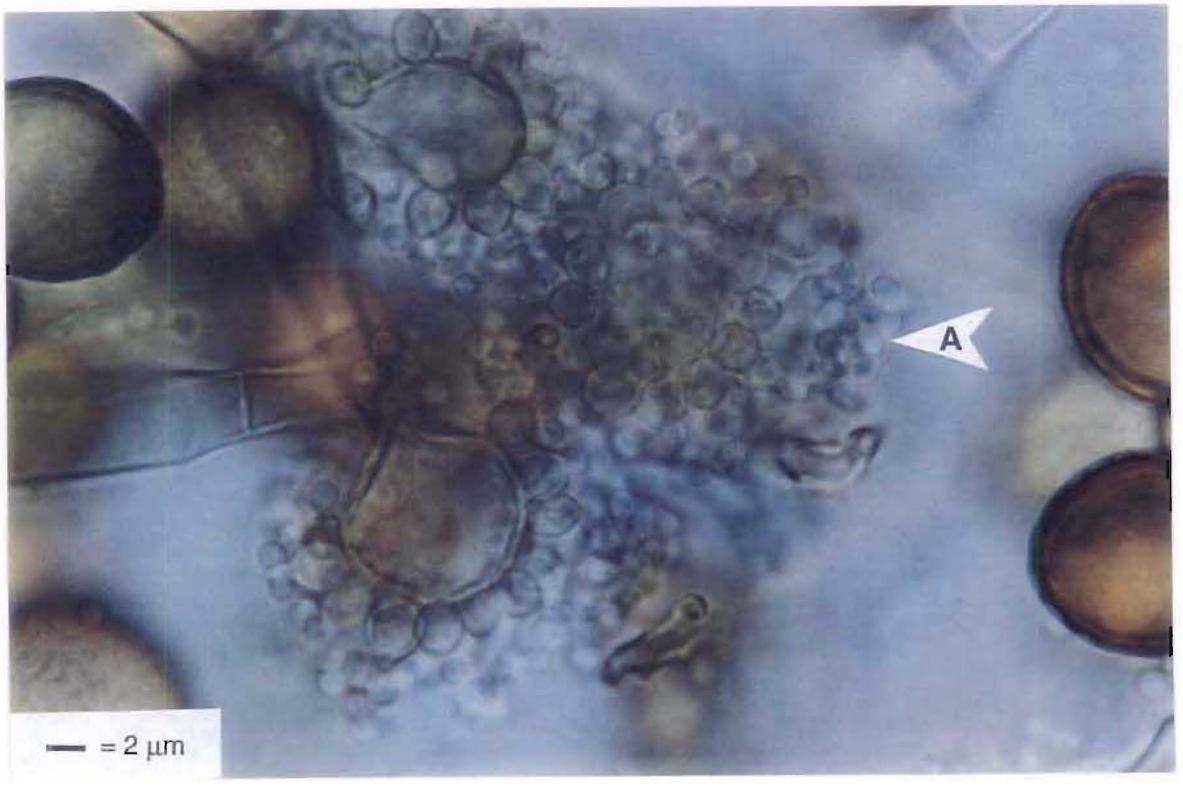


Figure 3.22 Phialospore state of *Acremoniella atra*, Phialospores (A), conidium (B).

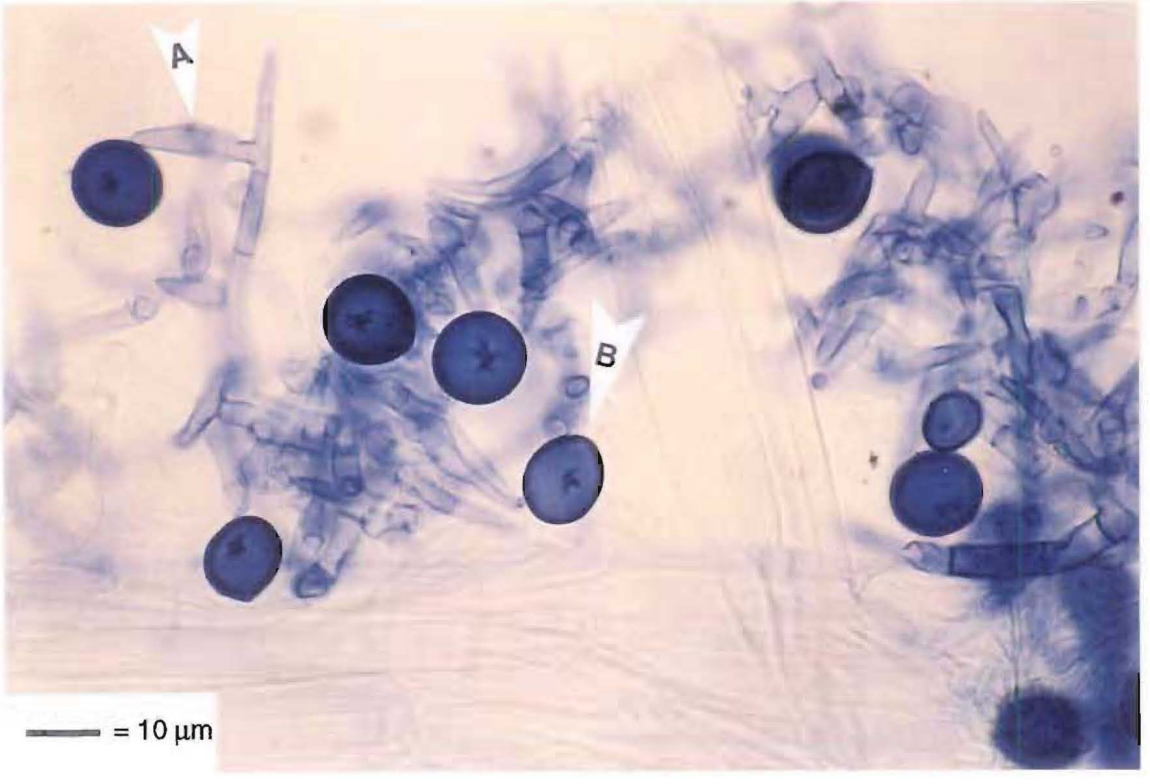


Figure 3.23 *Acremoniella atra* sporulating on the surface of an infected Yorkshire fog root, tapered conidiogenous cell (A), smooth ovoid conidium (B).

Regression analysis was undertaken to assess the relationship between visible disease symptoms and fungal root colonisation. The mean disease scores across all twelve species were averaged for each fungus (Table 3.11). The average disease scores were then plotted against the mean colonisation scores, which were also averaged across all twelve plant hosts. There was a significant positive correlation (R= 0.6282) between the disease symptoms observed on plant seedlings and the degree of hyphal colonisation of each fungus (Figure 3.24). Pathogenic fungi, which had high disease scores, were more often observed to invade root tissues systemically than non-pathogenic fungi. Thus results showed that systemic invasion of root tissues caused increased damage to inoculated seedlings. Most non-pathogenic fungi invaded roots only superficially, and therefore had both low colonisation and disease scores.

Table 3.11 Mean disease scores and colonisation scores, averaged for all twelve host plants.

Fungal Species	Disease Score	Colonisation score
Acremoniella atra	1.18	1.67
Acremonium curvulum	1.61	1.33
Acremonium strictum	3.14	1.33
Arthrinium arundinis	2.01	1.67
Aspergillus niger	0.75	0.92
Aspergillus ustus	0.94	0.92
Bimuria novae zelandiae	0.76	0.42
Botrytis cinerea	3.86	1.58
Chaetomium funicola	0.81	0.67
Chaetomium globosum	0.67	0.82
Clasterosporium	2.44	1.17
Codinaea fertilis	2.25	1.50
Colletotrichum	1.00	1.17
Curvularia trifolii	1.75	1.33
Cylindrocarpon destructans	2.74	1.67
Cylindrocladium scoparium	4.45	2.75
Dactylaria acerosa	0.96	0.83
Dreschlera dematioidea	3.13	2.17
Fusarium acuminatum	4.58	2.25
Fusarium avenaceum	4.94	2.67
Fusarium crookwellense	4.57	2.58
Fusarium culmorum	4.36	2.67
Fusarium equiseti	3.18	1.08
Fusarium graminum	2.08	1.50
Fusarium oxysporum	3.65	2.33
Fusarium sambucinum	3.54	2.08
Fusarium solani	3.11	1.67
Fusarium tricinctum	4.75	2.50
Gliocladium roseum	2.92	1.50
Gongronella butleri	0.31	0.92
Idriella bolleyi	3.09	1.33
Mariannaea elegans	0.25	1.00
Metarhizium anisopliae	0.03	0.33

Fungal species	Disease score	Colonisation score
Mortierella alpina	0.64	0.17
Mortierella elongata	0.15	0.58
Mortierella gamsii	2.63	0.91
Mortierella globulifera	0.95	0.75
Myrothecium verrucaria	3.04	0.83
Paecilomyces carneus	0.49	0.83
Paecilomyces lilacinus	1.75	0.92
Paecilomyces marquandii	0.35	0.92
Penicillium brevicompactum	1.28	0.92
P. chrysogenum	0.79	1.00
P. decumbens	0.40	0.75
P. griseofulvum	0.44	0.67
P. janczewskii	0.89	1.00
P. janthinellum	1.41	1.08
P. oxalicum	1.05	0.75
P. simplicissimum	1.31	1.08
P. variabile	0.78	0.83
Periconia macrospinosa	1.72	2.08
Phymatotrichum omnivorum	3.04	1.83
Pithomyces chartarum	0.80	0.45
Plectosporium tabacinum	2.75	1.33
Preussia aemulans	2.38	1.33
Ramichloridium schultzeri	1.32	1.00
Rhizoctonia solani	4.53	1.81
Sordaria fimicola	1.07	0.75
Tetraploa aristata	0.72	0.67
Thielaviopsis basicola	2.21	1.67
Thozetella tocklaiensis	0.57	0.92
Tricellula	0.61	0.75
Trichoderma hamatum	2.11	0.83
Trichoderma harzianum	2.59	0.42
Trichoderma koningii	1.98	0.50
Trichoderma polysporum	2.29	0.67
Trichoderma viride	1.76	0.08
Trichosporon cutaneum	0.36	0.75
Verticicladiella	0.56	1.00
Verticillium chlamydosporium	0.54	0.92

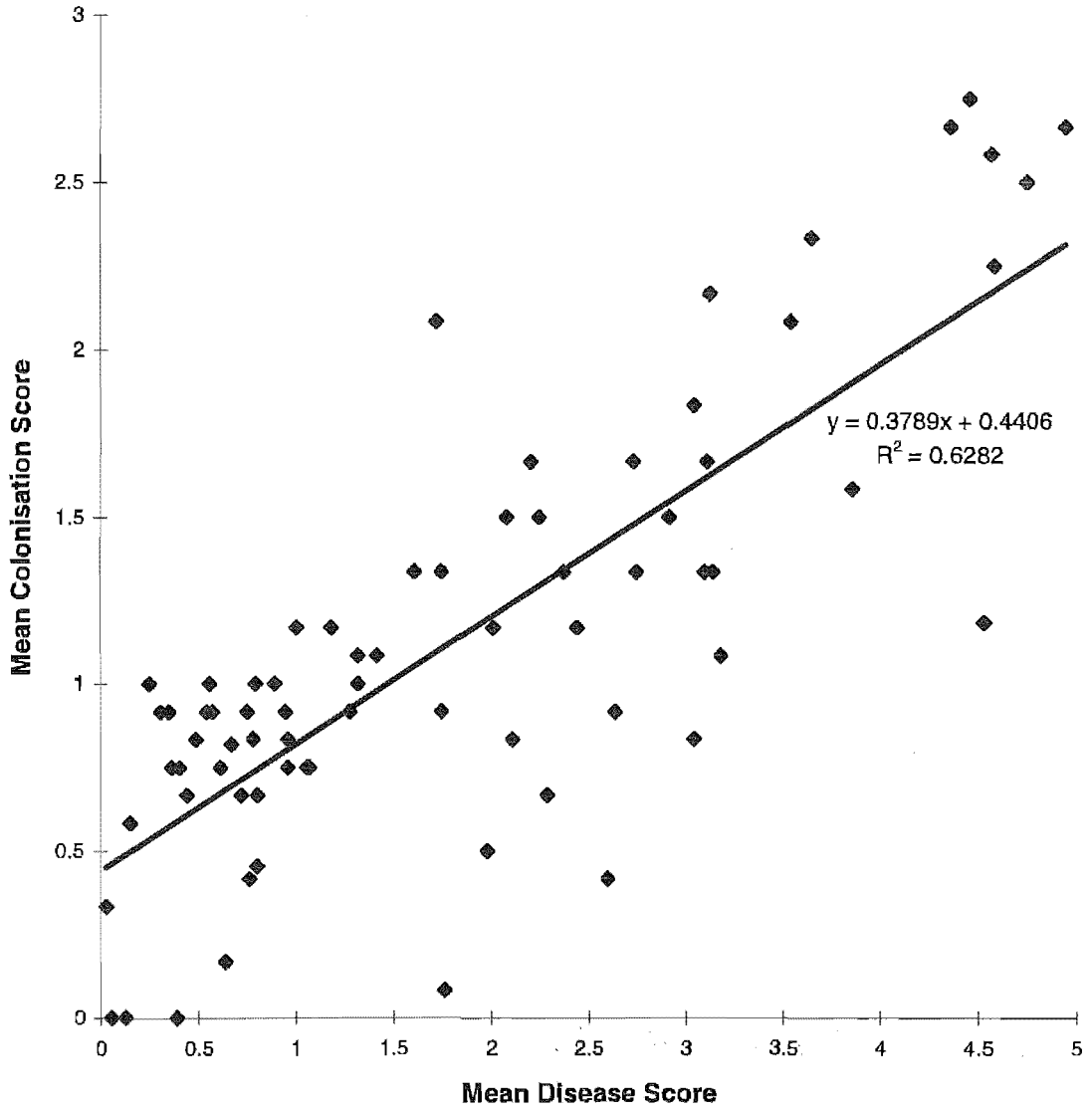


Figure 3.24 Correlation between mean disease scores and mean colonisation scores of 70 fungal species averaged across the seedlings of twelve pasture species.

3.3.1.(c) Light and Transmission Electron Microscopy (TEM) of infected root tissue.

Pathogens:

(i) *Codinaea fertilis*

Both inter and intracellular invasion of the epidermis and cortex by hyphae of *C. fertilis,* was observed in thin sections of white clover and subterranean clover under the light microscope. Intracellular infection cushions were observed in epidermal cells. Many of the infected epidermal cells of both clover species were completely disrupted, with localised cell wall degradation (Figure 3.25). Only intercellular hyphal invasion was seen in ryegrass sections, but some cell walls of infected epidermis cells had also been disrupted.

(ii) *Cylindrocarpon destructans*

Inter and intracellular hyphal invasion of epidermal and cortical cells of subterranean clover by *C. destructans* was observed using light microscopy (Figure 3.26). An intrahyphal hypha was observed inside an infected cortex cell (Figure 3.27). This was a simple intrahyphal hypha, in that it contained a single hypha growing within an existing hypha.

(iii) *Cylindrocladium scoparium*

This pathogen systemically invaded all root tissues of white clover and subterranean clover. Hyphal invasion was both inter and intracellular, and light microscopy also revealed that there was widespread disintegration of the cell walls of epidermal and cortical cells. Often this disintegration had occurred in uninfected cells suggesting the production of extracellular phytotoxins. An invasive hypha was observed to have actively penetrated the cell wall between two cortex cells (Figure 3.28).

(iv) *Fusarium crookwellense*

Systemic inter- and intracellular hyphal invasion by *F. crookwellense* was observed in all three hosts sampled (white clover, subterranean clover and ryegrass). Hyphae had penetrated epidermal, cortical and vascular cells causing severe and widespread cell wall disruption (Figure 3.29, 3.30)

(v) *Fusarium culmorum*

Systemic inter and intracellular hyphal invasion was also observed in subterranean clover root cells infected by *F. culmorum.* There was widespread cell wall disruption of the epidermis, including cells which appeared to be uninfected (Figure 3.31, 3.32).

(vi) *Mortierella gamsii*

Hyphae of *M. gamsii* were observed to invade the epidermis and cortex of subterranean clover by penetrating both lateral and longitudinal intercellular spaces (Figure 3.31). Invading hyphae were confined to the intercellular spaces but were observed to widen the space between cells. This fungus may have been able to penetrate intercellular spaces by the production of enzymes as the disruption and separation of cell walls was observed at some distance from the penetrating hyphae (Figure 3.33).

(vii) *Myrothecium verrucaria*

Inter and intracellular invasion of the epidermis of white clover was observed and the production of toxins may have been involved as the cell walls of uninfected epidermis and even some cortex cells were severely disrupted. Some root sections devoid of any hyphae were still observed to have degraded cell walls (Figures 3.34, 3.35)

Mild or non pathogens:

(i) *Bimuria novae zealandiae*

Intercellular hyphal invasion was observed in the epidermal cells of subterranean clover. Infection cushions were present at infection points on the epidermal cell wall, however infected tissue was still intact and there was no cell wall disruption in any root section.

(ii) Sterile dark group 6

Hyphal invasion of subterranean clover and ryegrass was by intercellular penetration through the epidermal wall. Hyphae were observed extending perpendicularly to the root axis, along the intercellular spaces into the outer cortex, where there was some intracellular hyphal invasion. Monilioid hyphae were also observed in cells (Figure 3.36).

Observations using TEM showed that hyphae of both pathogenic and non-pathogenic fungi invaded epidermal and cortical cells as well as the intercellular spaces. Of particular note was the observation of intrahyphal hyphae of *Cylindrocarpon destructans* not previously reported. Intrahyphal hyphae have been reported in a number of fungi including the vesicular arbuscular mycorrhizal fungus *Glomus fasiculatum* (Thaxter) Gerdemann & Trappe in the roots of white clover. (Lim *et al.* 1983). A number of hypotheses have been proposed for the proliferation of intrahyphal hyphae, and include the limitation of space, avoiding toxic metabolites or they may develop when initial infection hyphae become damaged and are then utilised by the fungus for re-establishment into previously colonised spaces (Lim *et al.* 1983).

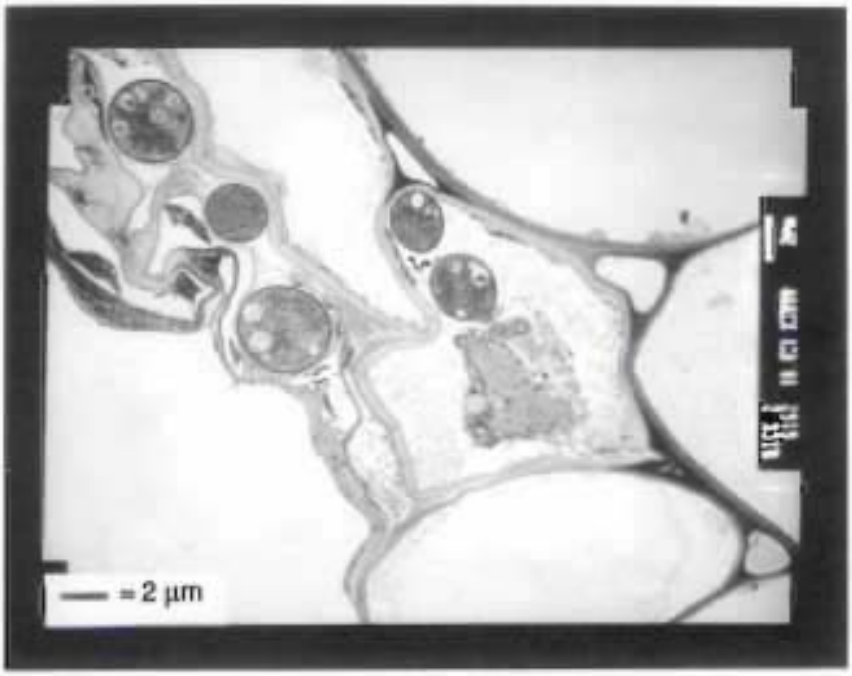


Figure 3.25 TEM transverse section (TS) root of subterranean clover showing (A) intercellular and (B) intracellular infection of *Codinaea fertilis* in the epidermis. (Note the severe cellular disruption caused by hyphal colonisation).

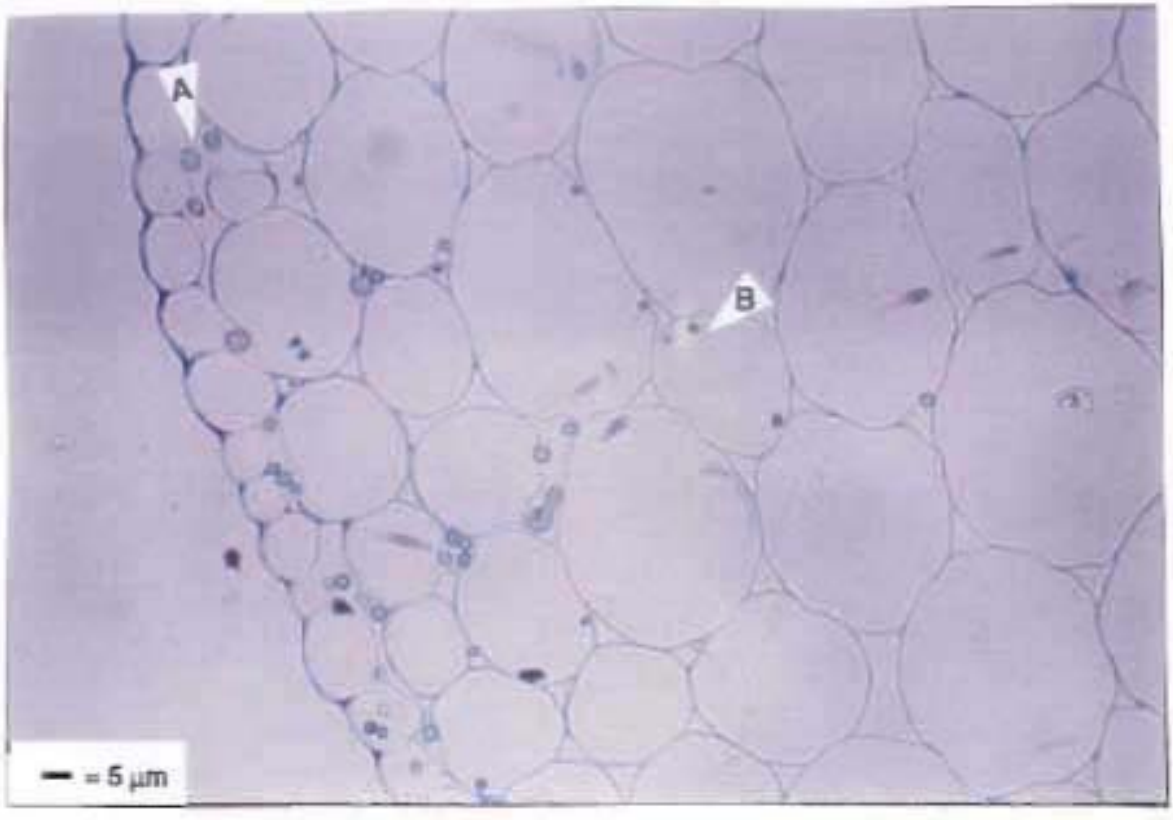


Figure 3.26 TS root of subterranean clover showing (A) intercellular and (B) intracellular infection by hyphae of *Cylindrocarpon destructans* of the epidermis and cortex.

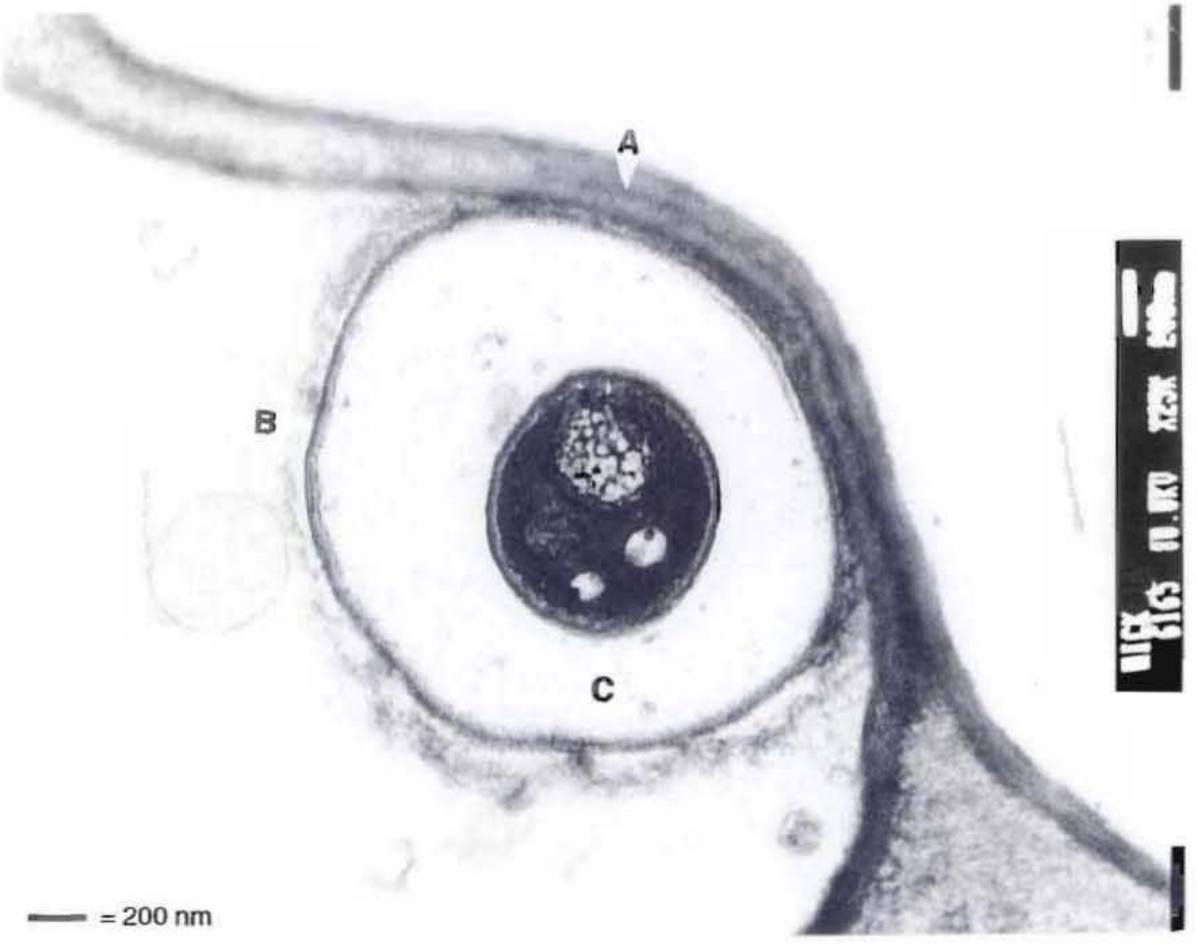


Figure 3.27 TEM TS of hypha of *Cylindrocarpon destructans* in the cortex of subterranean clover showing intrahyphal hyphae. (A) Host cell wall, (B) parent intracellular hypha, (C) intrahyphal hypha.

Figure 3.28 TEM TS root of subterranean clover showing (A) the intracellular hyphal penetration of *Cylindrocladium scoparium* through (B) a cortical cell wall.

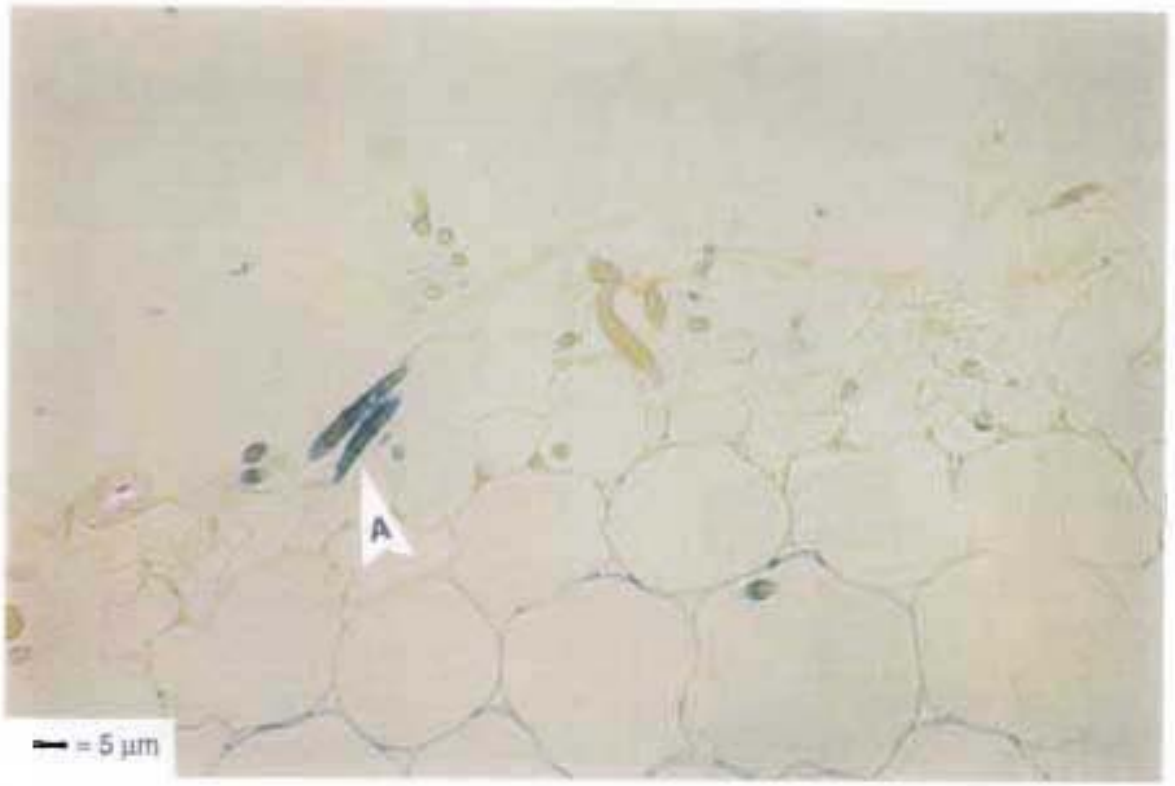


Figure 3.29 TS root of the epidermis and outer cortex of white clover showing the colonisation by hyphae of *Fusarium crookwellense*. The fungus has produced (A) macroconidia on infected root tissues.

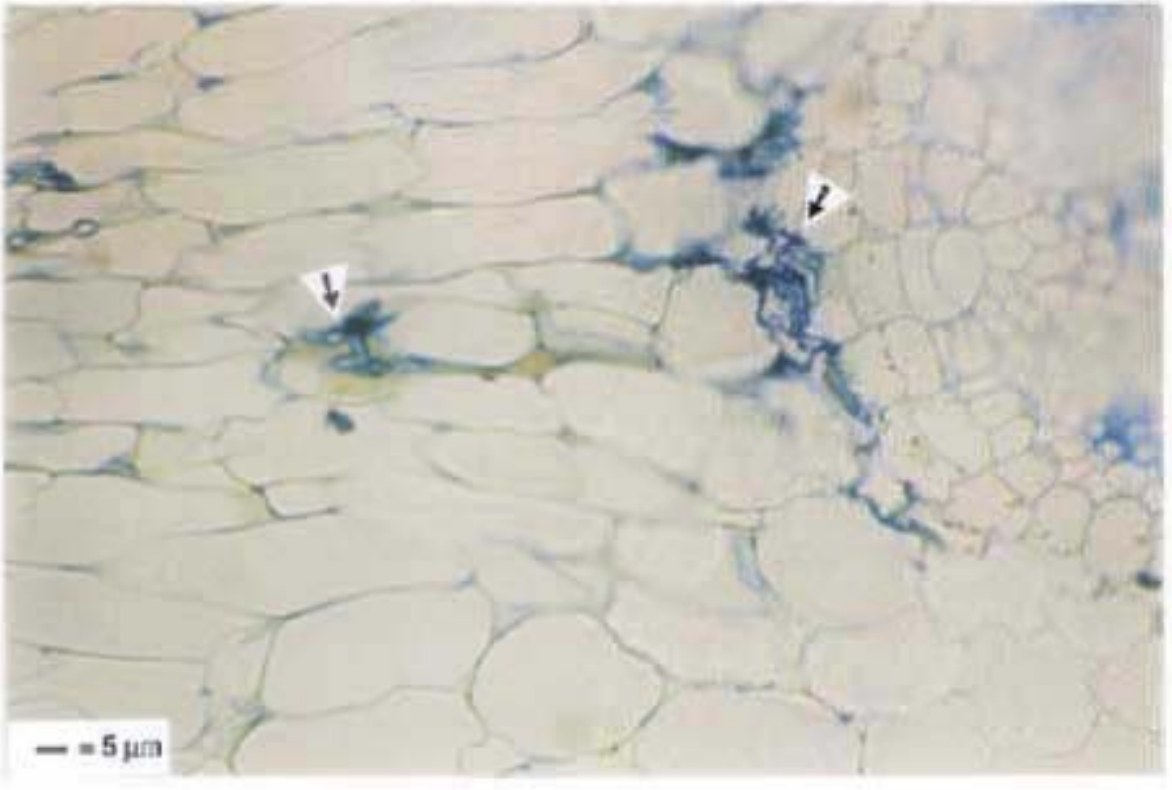


Figure 3.30 TS root of the inner cortex and vascular tissues of white clover by *F. crookwellense* showing the systemic hyphal invasion of white clover.

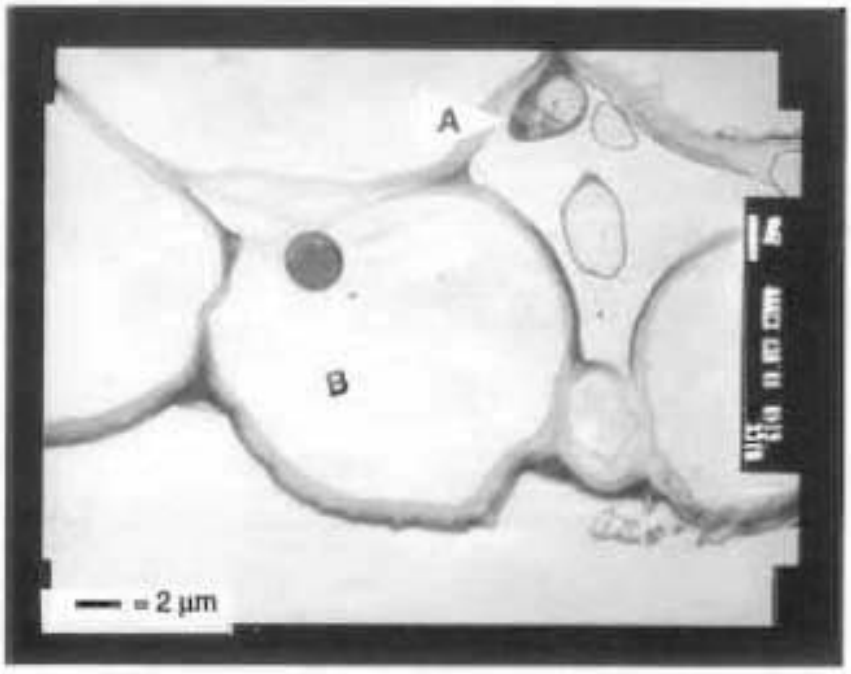


Figure 3.31 TEM TS of white clover root infected with *Fusarium culmorum*. Both intercellular (A) and intracellular (B) hyphal invasion has occurred.

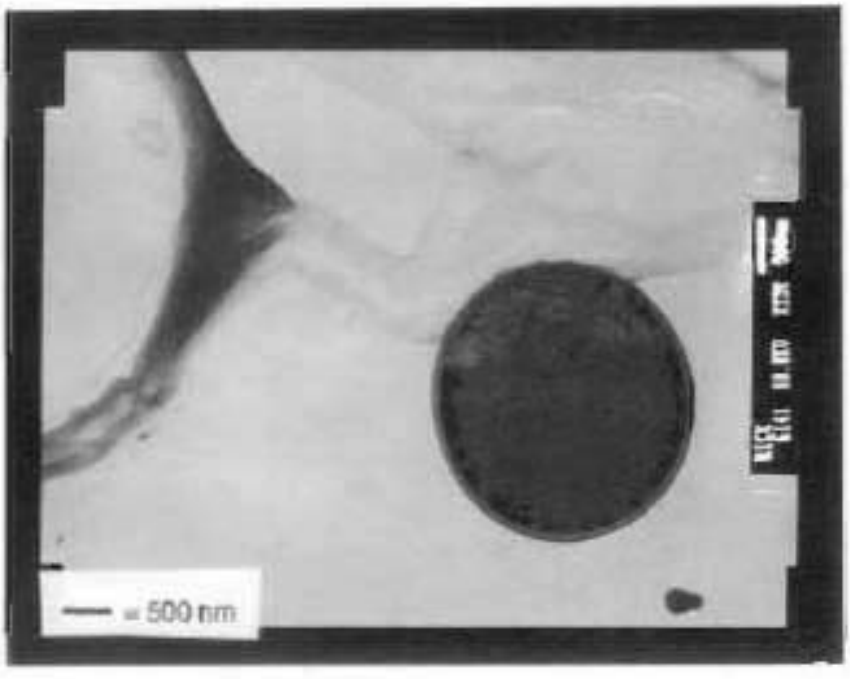


Figure 3.32 TEM TS of white clover root showing epidermal cell wall degradation associated with an intracellular hypha of *F. culmorum*.

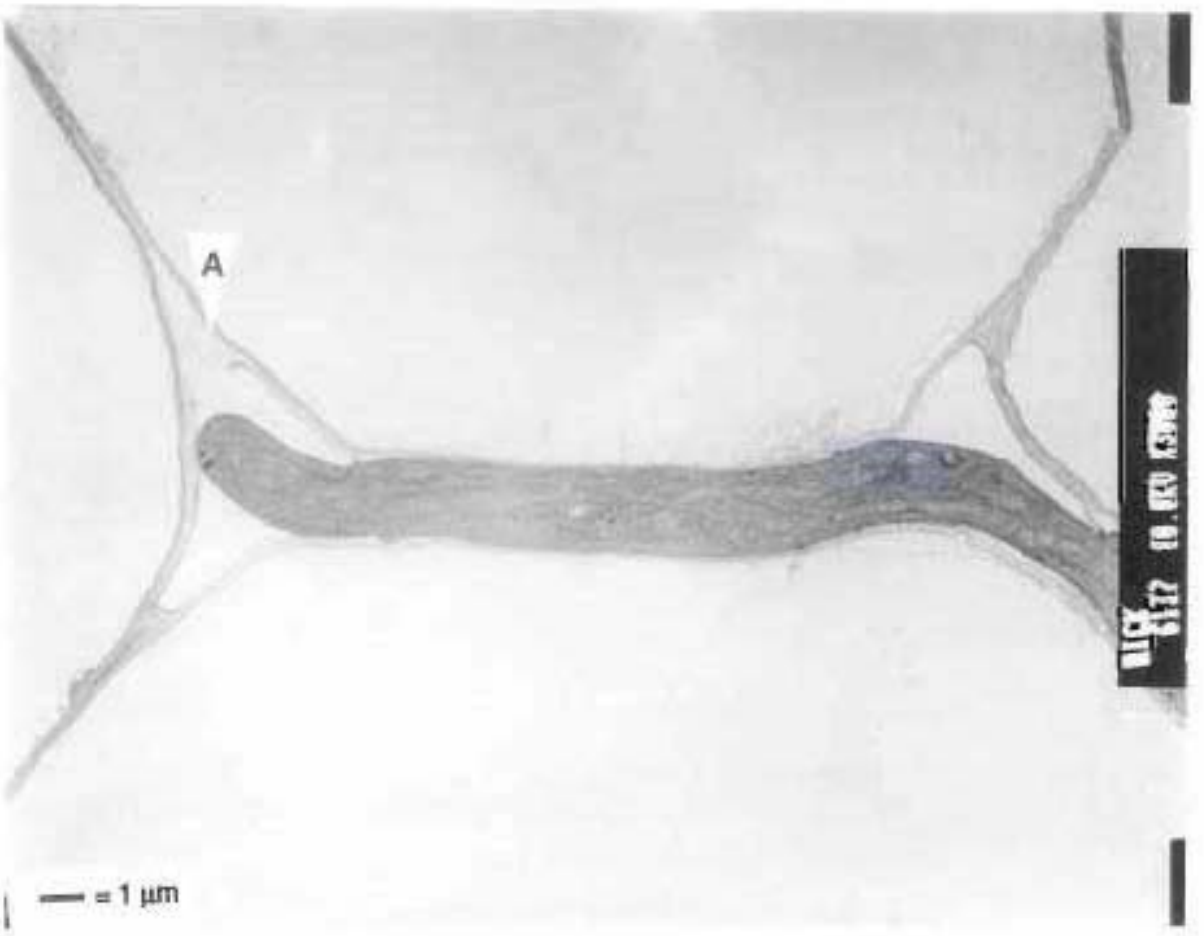


Figure 3.33 TEM TS of subterranean clover root showing intercellular penetration of (A) cortex cells by a hypha of *Mortierella gamsii* (B).

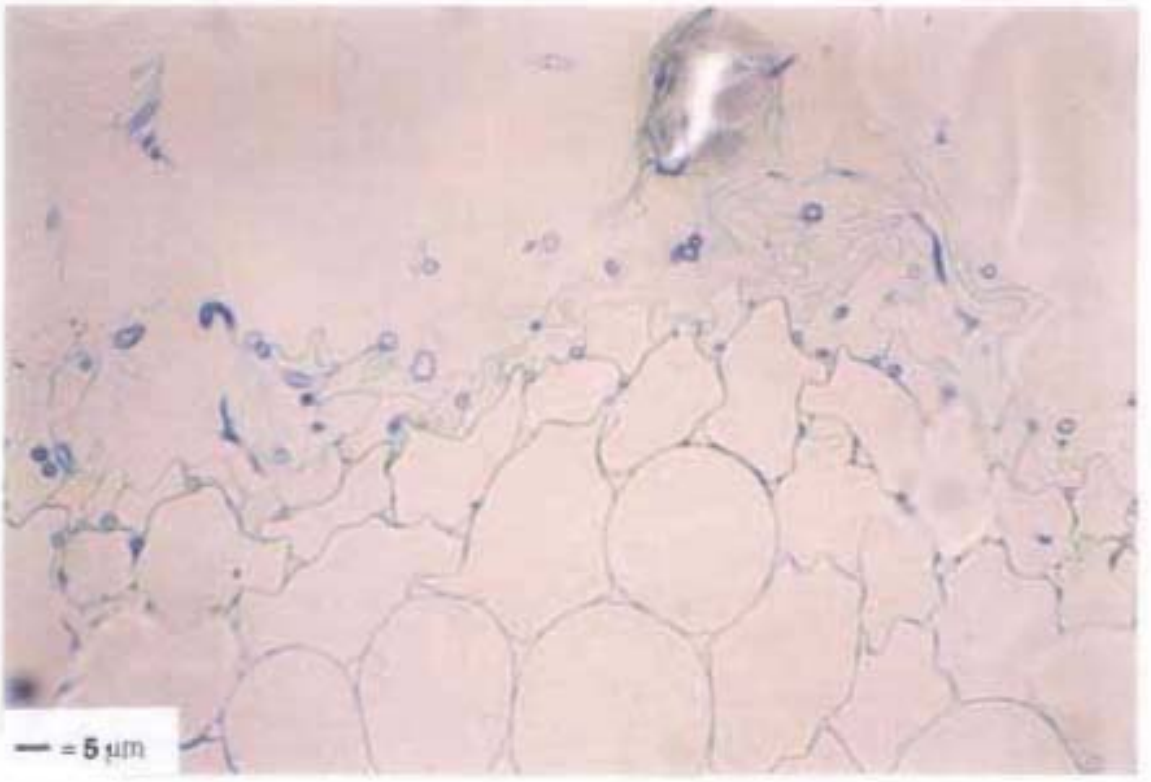


Figure 3.34 TS white clover root showing epidermal cellular disruption caused by the infection of *Myrothecium verrucaria*.

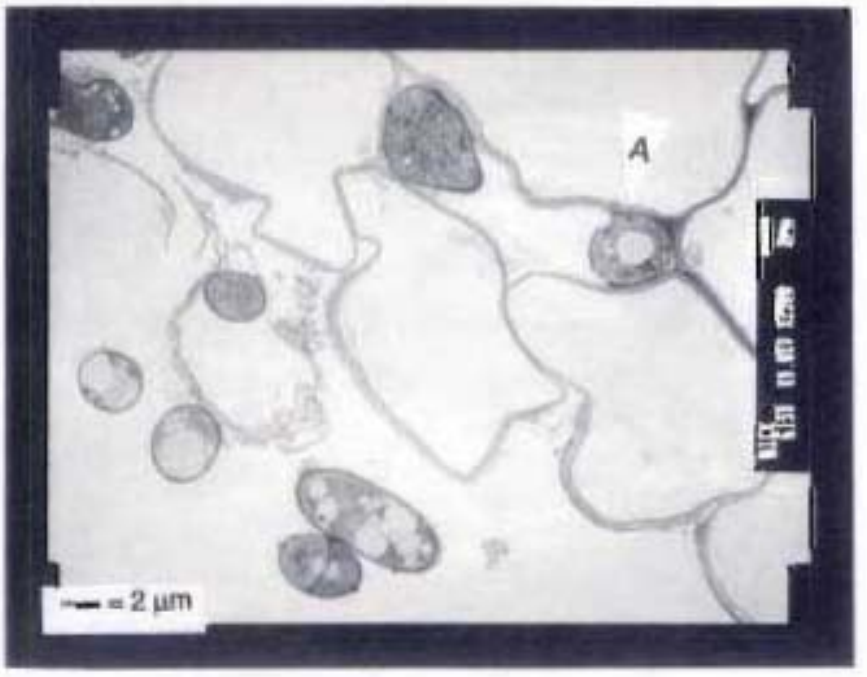


Figure 3.35 TEM TS white clover root showing epidermal cellular disruption caused by invasion of *Myrothecium verrucaria*. Intracellular hyphal invasion (A), Cell wall degradation (B).

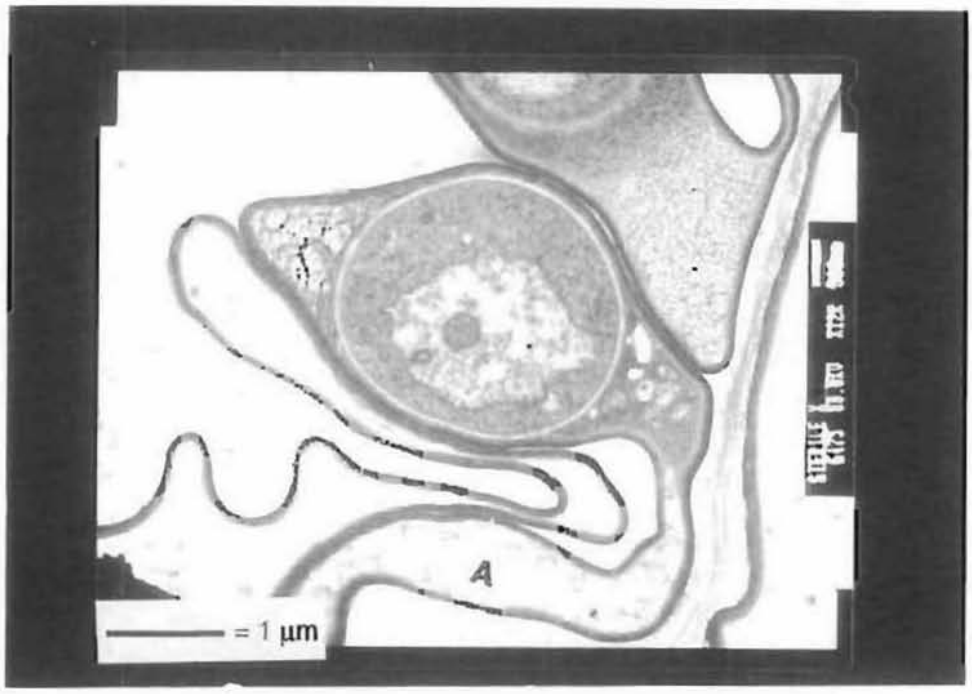


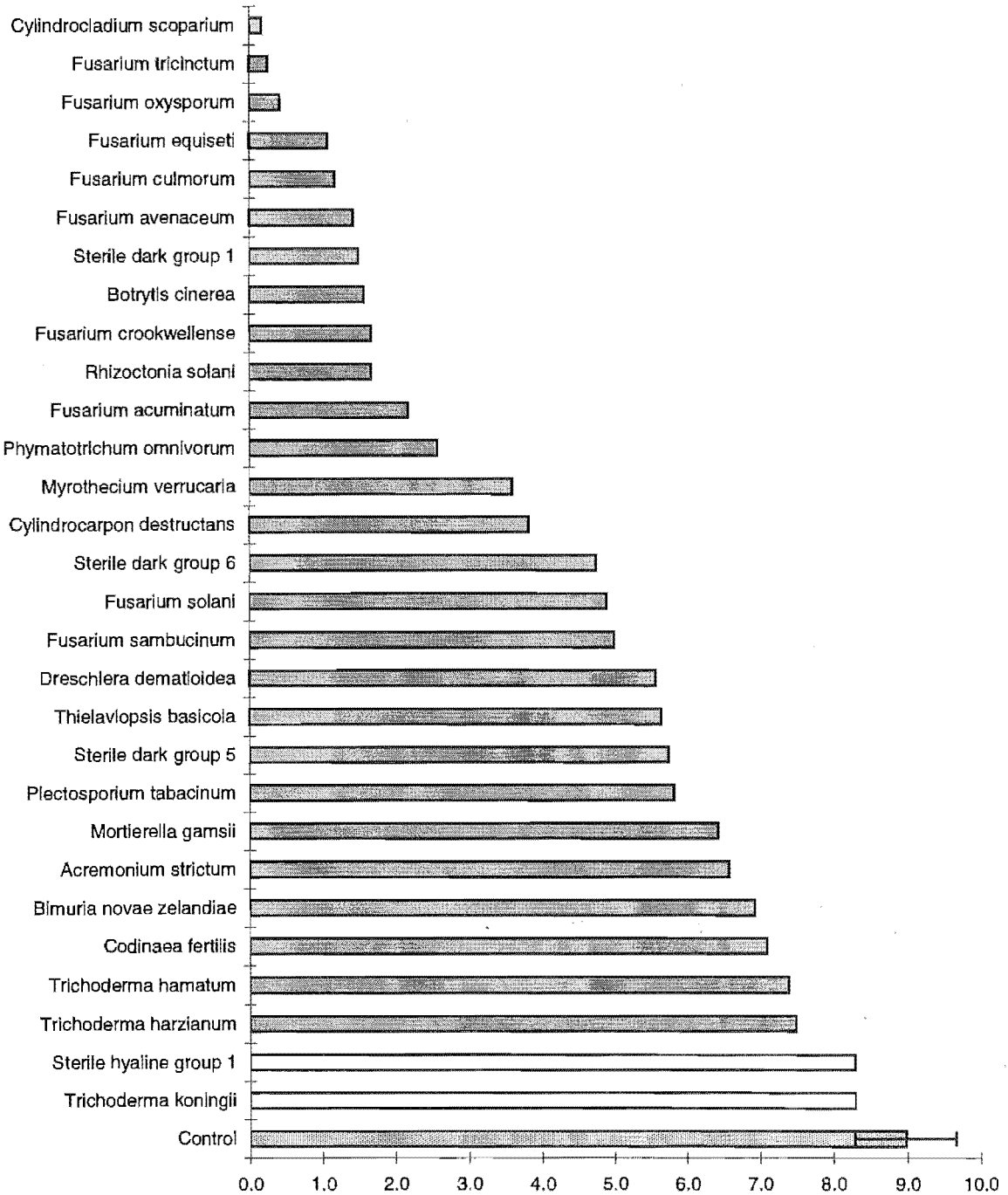
Figure 3.36 TEM TS of subterranean clover showing the intracellular hyphal invasion by sterile dark group 6. Cell walls (A) have been distorted by swollen monilioid hyphae.

3.3.2 EFFECT OF ROOT-COLONISING FUNGI ON THE EMERGENCE OF PASTURE SPECIES.

3.3.2.(a) Legume seedling emergence

All 29 fungal treatments, excluding *Trichoderma koningii* and sterile hyaline group 1 (SHG 1), caused significant ($P<0.05$) reduction in the mean seed emergence of legume plants (Figure 3.37). This result demonstrates these plant root pathogens are also virulent seed pathogens, although they may have killed germinated seedlings before emergence. *Cylindrocladium scoparium, Botrytis cinerea, Rhizoctonia solani* (Figure 3.38), and *Fusarium* spp., caused severe reduction of legume seed emergence, and was significantly lower than other fungal treatments. Three sterile dark fungi, sterile dark group 1 (SDG 1), sterile dark group 5 (SDG 5) and sterile dark group 6 (SDG 6), were all pathogenic and reduced legume seed emergence, but sterile hyaline group 1 did not reduce seedling emergence.

Emergence of white clover seedlings was significantly reduced by all but three fungal isolates (Table 3.12), and *B. cinerea* killed all inoculated seed. *Bimuria novae zelandiae* was found to be non-pathogenic to white clover legume seedlings using the axenic Petri plate method, but reduced seed emergence in this test. Red clover seed emergence was reduced by 23 fungal isolates including *C. scoparium, F. tricinctum, F. oxysporum,* and *F. equiseti* (Figure 3.39) which killed all seedlings (Table 3.12). Subterranean clover seed emergence was the worst affected by fungal inoculation as nine fungi killed all seeds (Table 3.12), and all remaining species, excluding *B. novae zelandiae, M. gamsii* and SHG 1, also significantly reduced seedling emergence. Lotus seedling emergence was the least affected of the legumes, with nine species causing no significant reduction in emergence compared with controls. However, *C. scoparium, F. culmorum, F. acuminatum,* and *F. tricinctum* were observed to kill all lotus seedlings (Table 3.12, Figure 3.40) and a further 16 fungal treatments caused a lower emergence than the controls.



Error bar = SEM 0.689
Shaded bars indicate fungal treatments which significantly reduced seed emergence compared to the control $P<0.05$

Figure 3.37 Mean seedling emergence of four legume species inoculated with root-colonising fungi.

Table 3.12 Mean emergence of white clover, red clover , subterranean clover and lotus seed inoculated with root-colonising fungi.

White Clover	1*	Red Clover	2*	Subterranean clover	3*	Lotus	4*
Control	**8.8**	*T. koningii*	**9.0**	**Control**	**8.3**	*T. koningii*	**10.0**
Thielaviopsis basicola	**8.3**	**Control**	**8.8**	*M. gamsii*	**8.0**	*D. dematioidea*	**9.7**
SHG 1	**8.3**	*C. fertilis*	**8.7**	*B. novae zelandiae*	**8.0**	**Control**	**9.7**
Trichoderma koningii	**8.0**	*P. tabacinum*	**8.3**	SHG 1	**7.3**	SHG 1	**9.3**
Trichoderma harzianum **	**7.7**	*F. sambucinum*	**8.0**	*T. harzianum*	**6.3**	*T. hamatum*	**9.3**
Trichoderma hamatum	**7.3**	*M. gamsii*	**8.0**	*T. hamatum*	**6.3**	*A. strictum*	**9.3**
Fusarium acuminatum	**7.0**	SHG 1	**7.7**	*A. strictum*	**6.3**	*T. harzianum*	**9.0**
SDG 5	**7.0**	*F. solani*	**7.0**	*T. koningii*	**5.7**	*M. gamsii*	**9.0**
Plectosporium tabacinum	**6.3**	*T. harzianum*	**7.0**	*C. fertilis*	**5.0**	SDG 6	**8.7**
Codinaea fertilis	**6.0**	*T. hamatum*	**6.7**	*T. basicola*	**4.3**	*C. fertilis*	**8.7**
Acremonium strictum	**6.0**	*B. novae zelandiae*	**6.7**	*D. dematioidea*	**4.0**	SDG 5	**8.0**
Fusarium sambucinum	**5.0**	*D. dematioidea*	**6.3**	*P. tabacinum*	**4.0**	*B. novae zelandiae*	**8.0**
Bimuria novae zelandiae	**5.0**	*T. basicola*	**5.7**	SDG 5	**3.7**	*F. sambucinum*	**7.0**
Fusarium avenaceum	**4.7**	*M. verrucaria*	**4.7**	*P. omnivorum*	**3.0**	*C. destructans*	**6.7**
Cylindrocarpon destructans	**4.0**	*A. strictum*	**4.7**	*F. solani*	**3.0**	*F. solani*	**6.3**
SDG 6	**4.0**	SDG 6	**4.3**	SDG 6	**2.0**	*M. verrucaria*	**5.3**
Fusarium solani	**3.3**	SDG 5	**4.3**	*M. verrucaria*	**1.7**	*B. cinerea*	**5.0**
Phymatotrichum omnivorum	**3.0**	SDG 1	**4.0**	*R. solani*	**1.7**	*P. tabacinum*	**4.7**
Fusarium culmorum	**3.0**	*C. destructans*	**3.3**	*C. destructans*	**1.3**	*T. basicola*	**4.3**
Myrothecium verrucaria	**2.7**	*F. crookwellense*	**2.7**	SDG 1	**1.0**	*F. equiseti*	**4.0**
Dreschlera dematioidea	**2.3**	*F. acuminatum*	**1.7**	*F. crookwellense*	**0.3**	*P. omnivorum*	**4.0**
Rhizoctonia solani	**2.0**	*F. culmorum*	**1.7**	*F. equiseti*	**0.0**	*R. solani*	**2.3**
Fusarium crookwellense	**1.7**	*B. cinerea*	**1.3**	*F. oxysporum*	**0.0**	*F. crookwellense*	**2.0**
Fusarium tricinctum	**1.0**	*F. avenaceum*	**0.7**	*B. cinerea*	**0.0**	*F. oxysporum*	**1.0**
SDG 1	**0.7**	*R. solani*	**0.7**	*F. tricinctum*	**0.0**	SDG 1	**0.3**
Fusarium oxysporum	**0.7**	*P. omnivorum*	**0.3**	*F. acuminatum*	**0.0**	*F. avenaceum*	**0.3**
Mortierella gamsii	**0.7**	*F. equiseti*	**0.0**	*F. sambucinum*	**0.0**	*F. tricinctum*	**0.0**
Cylindrocladium scoparium	**0.7**	*F. oxysporum*	**0.0**	*F. culmorum*	**0.0**	*F. acuminatum*	**0.0**
Fusarium equiseti	**0.3**	*F. tricinctum*	**0.0**	*F. avenaceum*	**0.0**	*F. culmorum*	**0.0**
Botrytis cinerea	**0.0**	*C. scoparium*	**0.0**	*C. scoparium*	**0.0**	*C. scoparium*	**0.0**

*** 1,2,3,4 Mean seedling emergence (number of seeds germinated) across three replicates.**

**** Shaded cells represent treatments which are significantly lower than the controls (P<0.05)**

Figure 3.38 Subterranean clover seed inoculated with *Rhizoctonia solani* (right pot, uninoculated control treatment with complete seedling emergence (left pot).

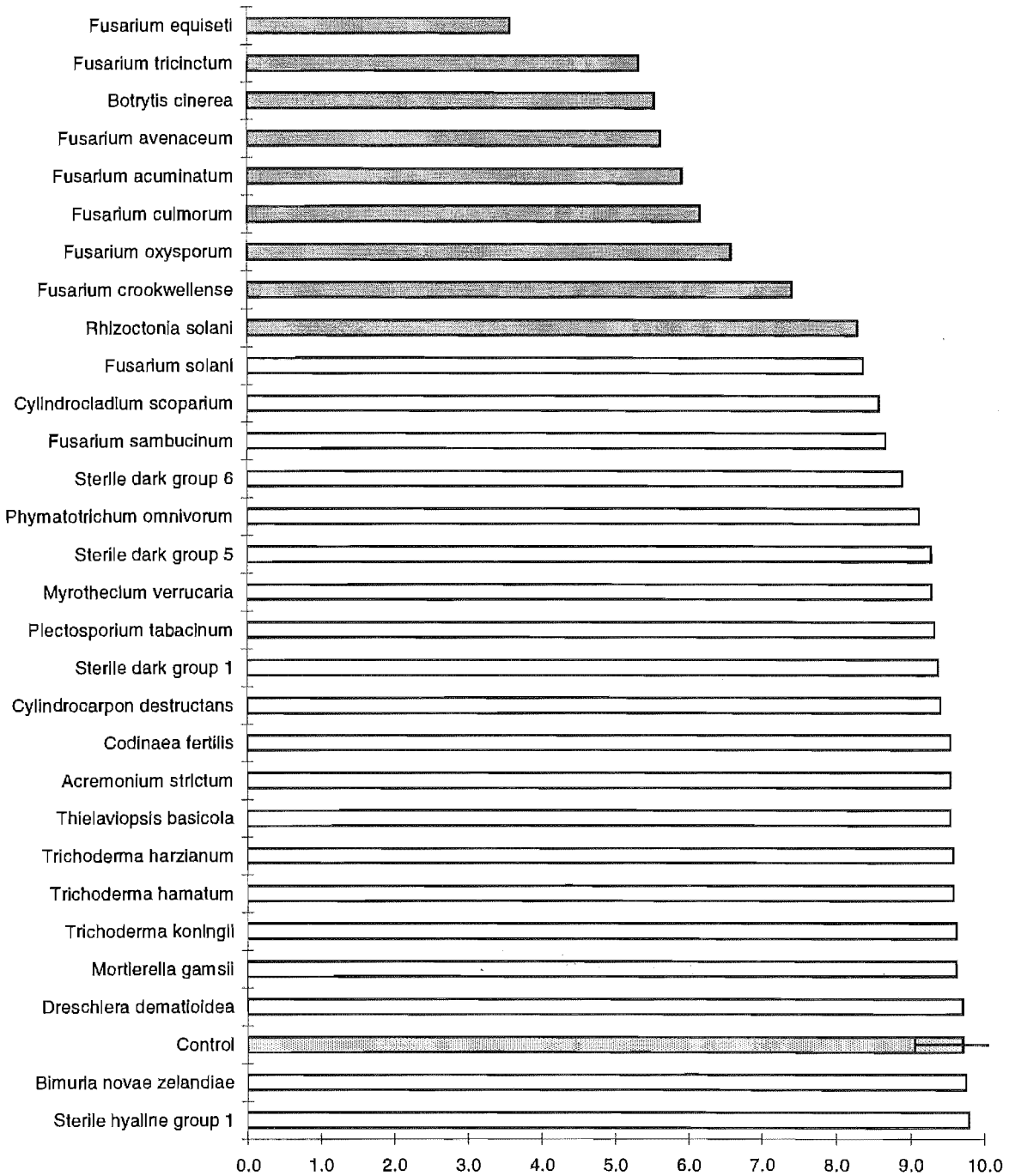
Figure 3.39 100% mortality of red clover seed inoculated with *Fusarium equiseti*.

Figure 3.40 Lotus seed inoculated with *Fusarium avenaceum* (right pot), uninoculated control treatment with complete seedling emergence (left pot).

3.3.2.(b) Grass seedling emergence

In contrast to the test with legumes, only *Fusarium* spp., *B. cinerea* and *R. solani* caused significant reduction in the mean seedling emergence of the eight grass species (Figure 3.41), as the mean grass emergence was over 90% which was also the emergence of control seed (Table 3.13). *Fusarium equiseti* was pathogenic to all grasses and caused the greatest mean reduction of grass seedling emergence (Figure 3.42). *Fusarium tricinctum, F. avenaceum F. acuminatum,* and *F. culmorum* reduced mean grass seed emergence by up to 50%. Overall results show that grass seedlings were more resistant to fungal infection and disease than the legume seedlings.

Species of *Fusarium* and *B. cinerea* were pathogenic to seed of sweet vernal, browntop (Figure 3.43), timothy, and Yorkshire fog (Table 3.13). *Fusarium equiseti* was observed to cause the highest reduction in seed emergence of these four plants, particularly to browntop seeds where 100% mortality was observed. In addition to the pathogenic *Fusarium* and *Botrytis* treatments, *P. omnivorum, R. solani* and SDG 6, reduced sweet vernal emergence, *C. scoparium* and SDG 6 reduced browntop emergence, and *C. fertilis* and *P. tabacinum* reduced timothy seedling emergence (Table 3.13).



Error bar = SEM 0.652
Shaded bars indicate fungal treatments which significantly reduced seedling emergence compared to the control $P<0.05$

Figure 3.41 Mean seedling emergence of eight grass species inoculated with root-colonising fungi.

Table 3.13 Mean emergence of sweet vernal, browntop, timothy, Yorkshire fog seed inoculated with root-colonising fungi.

Sweet Vernal	1	Browntop	2	Timothy	3	Yorkshire Fog	4
Trichoderma koningii	9.7	SDG 1	10.0	*D. dematioidea*	10.0	SDG 1	10.0
Control	9.7	*M. verrucaria*	10.0	*M. verrucaria*	10.0	*D. dematioidea*	10.0
SDG 1	9.3	*R. solani*	10.0	SDG 5	10.0	*P. omnivorum*	10.0
SHG 1	9.3	*T. basicola*	10.0	*T. basicola*	10.0	*T. basicola*	10.0
Trichoderma hamatum	9.3	*T. harzianum*	10.0	*T. harzianum*	10.0	SHG 1	10.0
Bimuria novae zelandiae	9.3	SHG 1	10.0	*M. gamsii*	10.0	*P. tabacinum*	10.0
Acremonium strictum	9.3	*T. hamatum*	10.0	*B. novae zelandiae*	10.0	*M. gamsii*	10.0
Dreschlera dematioidea	9.0	*C. fertilis*	10.0	*T. koningii*	10.0	*B. novae zelandiae*	10.0
Thielaviopsis basicola	9.0	*M. gamsii*	10.0	**Control**	9.8	*C. destructans*	9.7
Trichoderma harzianum	9.0	*B. novae zelandiae*	10.0	SDG 1	9.7	*R. solani*	9.7
Codinaea fertilis	9.0	*D. dematioidea*	9.7	SHG 1	9.7	*T. hamatum*	9.7
Plectosporium tabacinum	9.0	*T. koningii*	9.7	*T. hamatum*	9.7	**Control**	9.5
Cylindrocladium scoparium	9.0	**Control**	9.7	*C. destructans*	9.3	*M. verrucaria*	9.3
Myrothecium verrucaria	8.7	*C. destructans*	9.3	SDG 6	9.3	SDG 5	9.3
Fusarium sambucinum	8.7	*F. solani*	9.3	*P. omnivorum*	9.3	*T. harzianum*	9.3
SDG 5	8.7	*F. sambucinum*	9.3	*F. sambucinum*	9.3	*A. strictum*	9.3
Mortierella gamsii	8.7	*P. omnivorum*	9.0	*R. solani*	9.3	*F. sambucinum*	9.0
Cylindrocarpon destructans	8.3	SDG 5	9.0	*C. scoparium*	9.3	*C. scoparium*	9.0
Phymatotrichum omnivorum	8.0	*P. tabacinum*	9.0	*A. strictum*	9.3	SDG 6	8.7
*Fusarium culmorum***	8.0	*A. strictum*	9.0	*F. solani*	8.7	*C. fertilis*	8.7
SDG 6	7.7	SDG 6	8.3	*C. fertilis*	8.7	*T. koningii*	8.7
Rhizoctonia solani	7.3	*F. crookwellense*	8.3	*P. tabacinum*	8.7	*F. tricinctum*	8.3
Fusarium oxysporum	7.0	*F. oxysporum*	7.7	*F. crookwellense*	7.3	*F. solani*	8.3
Botrytis cinerea	6.3	*F. acuminatum*	7.3	*F. acuminatum*	7.0	*F. acuminatum*	7.3
Fusarium solani	6.3	*F. culmorum*	7.3	*F. culmorum*	7.0	*F. crookwellense*	7.0
Fusarium crookwellense	5.7	*C. scoparium*	7.3	*F. oxysporum*	6.3	*F. culmorum*	6.7
Fusarium avenaceum	5.3	*F. tricinctum*	5.0	*F. tricinctum*	6.0	*F. oxysporum*	5.7
Fusarium tricinctum	2.3	*B. cinerea*	4.0	*B. cinerea*	5.3	*B. cinerea*	5.7
Fusarium acuminatum	1.7	*F. avenaceum*	2.3	*F. avenaceum*	4.3	*F. avenaceum*	5.0
Fusarium equiseti	1.0	*F. equiseti*	0.0	*F. equiseti*	2.3	*F. equiseti*	3.0

*** 1, 2, 3, 4 Mean seedling emergence (number of seeds germinated) across three replicates.**

**** Shaded cells represent treatments which are significantly lower than the controls (P<0.05)**

Figure 3.42 Seedling emergence of sweet vernal inoculated with *Fusarium equiseti* (left pot), uninoculated control treatment (right pot).

Figure 3.43 Seedling emergence of browntop inoculated with *Fusarium crookwellense* (left pot), uninoculated control treatment (right pot).

Emergence of tall fescue, ryegrass, cocksfoot and soft brome seedlings was also reduced by *Fusarium* spp. and *B. cinerea* (Table 3.14, Figures 3.44 - 3.45) while emergence of cocksfoot and soft brome seedlings was reduced by *R. solani* and *C. scoparium*, and SDG 1 reduced ryegrass emergence. *Fusarium tricinctum* was the most pathogenic fungus to both cocksfoot and soft brome, while *B. cinerea* and *F. culmorum* were the most pathogenic fungi to tall fescue and ryegrass. Tall fescue was the least affected grass host, as only six fungal treatments reduced emergence significantly.

Table 3.14 Mean emergence of tall fescue, ryegrass, cocksfoot and soft brome seed inoculated with root-colonising fungi.

Tall fescue	1*	Ryegrass	2	Cocksfoot	3	Soft brome	4
Cylindrocarpon destructans	10.0	*D. dematioidea*	10.0	SDG 6	10.0	*C. destructans*	10.0
SHG 1	10.0	*P. omnivorum*	10.0	SHG 1	10.0	*D. dematioidea*	10.0
Codinaea fertilis	10.0	*T. harzianum*	10.0	*C. fertilis*	10.0	*T. hamatum*	10.0
Mortierella gamsii	10.0	SHG 1	10.0	*B. novae zelandiae*	10.0	*C. fertilis*	10.0
Acremonium strictum	10.0	*T. hamatum*	10.0	*A. strictum*	10.0	*M. gamsii*	10.0
Dreschlera dematioidea	9.7	*C. fertilis*	10.0	**Control**	10.0	*A. strictum*	10.0
Bimuria novae zelandiae	9.7	*P. tabacinum*	10.0	*F. solani*	9.7	**Control**	10.0
Trichoderma koningii	9.7	*T. koningii*	10.0	SDG 5	9.7	SDG 6	9.7
SDG 1	9.3	**Control**	10.0	*T. harzianum*	9.7	SDG 5	9.7
Myrothecium verrucaria	9.3	*R. solani*	9.7	*T. koningii*	9.7	*T. koningii*	9.7
Thielaviopsis basicola	9.3	*T. basicola*	9.7	*C. destructans*	9.3	SDG 1	9.3
Trichoderma harzianum	9.3	*C. scoparium*	9.7	SDG 1	9.3	*T. harzianum*	9.3
Trichoderma hamatum	9.3	*B. novae zelandiae*	9.7	*D. dematioidea*	9.3	SHG 1	9.3
Plectosporium tabacinum	9.3	*C. destructans*	9.3	*T. basicola*	9.3	*P. tabacinum*	9.3
Control	9.0	*M. verrucaria*	9.3	*P. tabacinum*	9.3	*B. novae zelandiae*	9.3
Phymatotrichum omnivorum	8.7	SDG 6	9.3	*P. omnivorum*	9.0	*M. verrucaria*	9.0
SDG 5	8.7	SDG 5	9.3	*M. gamsii*	9.0	*P. omnivorum*	9.0
Cylindrocladium scoparium	8.7	*M. gamsii*	9.3	*M. verrucaria*	8.7	*T. basicola*	9.0
Rhizoctonia solani	8.3	*A. strictum*	9.3	*T. hamatum*	8.7	*F. sambucinum*	8.7
Fusarium crookwellense	8.3	*B. cinerea*	9.0	*C. scoparium*	7.7	*F. equiseti*	8.3
SDG 6	8.2	*F. tricinctum*	9.0	*F. sambucinum*	7.3	*F. solani*	8.3
Fusarium solani	8.0	*F. sambucinum*	9.0	*F. oxysporum*	6.0	*F. culmorum*	8.3
Fusarium sambucinum	8.0	*F. crookwellense*	9.0	*F. crookwellense*	5.7	*R. solani*	8.3
Fusarium avenaceum	8.0	*F. acuminatum*	8.7	*F. avenaceum*	5.0	*F. crookwellense*	8.0
*Fusarium equiseti***	7.3	*F. avenaceum*	8.7	*R. solani*	3.7	*C. scoparium*	8.0
Fusarium oxysporum	7.0	*F. solani*	8.3	*B. cinerea*	3.0	*F. oxysporum*	7.3
Fusarium tricinctum	7.0	SDG 1	8.0	*F. equiseti*	1.7	*F. acuminatum*	7.3
Fusarium culmorum	7.0	*F. oxysporum*	5.7	*F. acuminatum*	1.3	*F. avenaceum*	6.3
Fusarium acuminatum	6.7	*F. equiseti*	5.0	*F. culmorum*	0.7	*B. cinerea*	6.0
Botrytis cinerea	5.0	*F. culmorum*	4.3	*F. tricinctum*	0.3	*F. tricinctum*	4.7

*** 1, 2, 3, 4 Mean seedling emergence (number of seeds germinated) across three replicates.**

**** Shaded cells represent treatments which are significantly lower than the controls (P<0.05)**

Figure 3.44 Ryegrass seedling emergence after inoculation with *Fusarium equiseti* (left pot), uninoculated control treatment (right pot).

Figure 3.45 Soft brome seedling emergence after inoculation with *Fusarium oxysporum* (left pot), uninoculated control treatment (right pot).

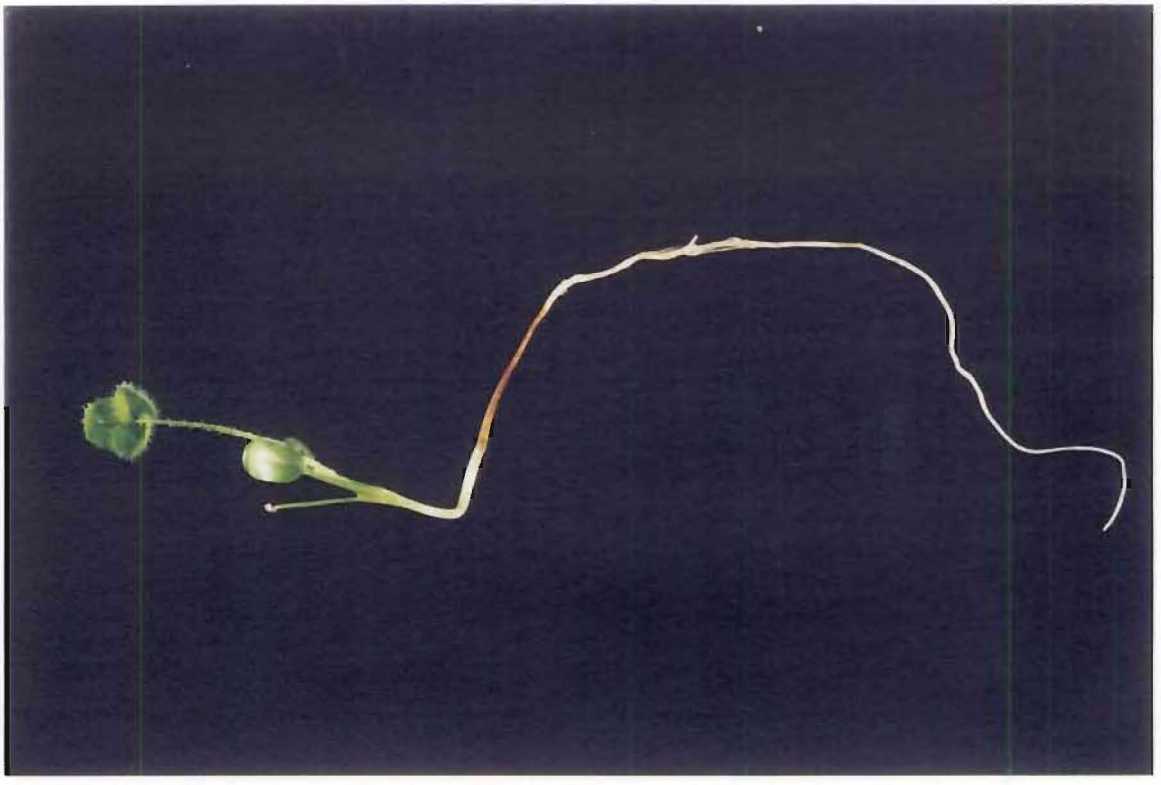


Figure 3.46 Disease lesion on a subterranean clover root infected by *Codinaea fertilis* after germination.

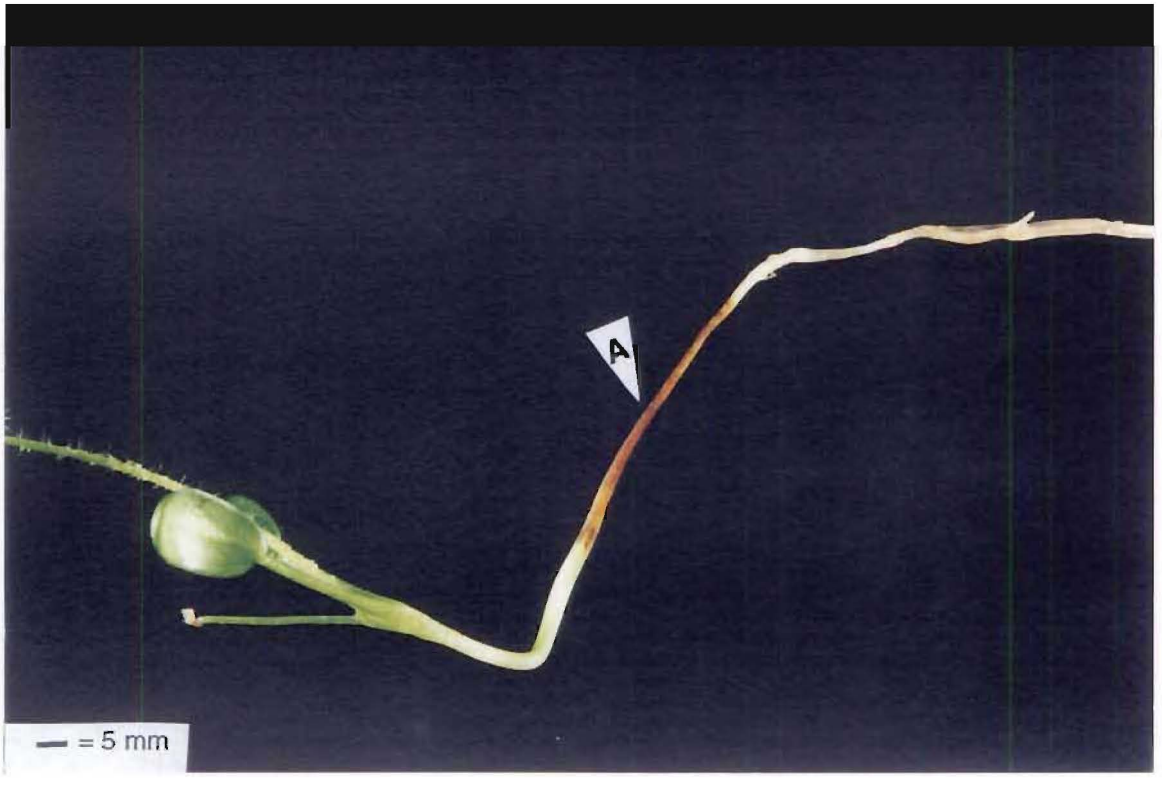


Figure 3.47 Disease lesion (A) on a subterranean clover root infected by *Codinaea fertilis* after germination.

Infection of seeds caused discoloration, softening and rot of the seed coat. hyphal colonisation of seeds and loss of seed turgor were also observed. Root disease symptoms were observed on most seedlings that germinated and survived to be harvested (Figures 3.46 - 3.49) and many were observed to have reduced root growth (Figure 3.50, 3.51). Mean disease scores were higher for the four inoculated legume hosts (Figure 3.52) than the eight inoculated grass hosts (Figure 3.53). As there were fewer fungi which affected grass seed emergence, more grass seedlings were harvested at the end of this trial than legume seedlings and many of these fungi were observed to infect the seedling roots after germination. All hosts were inoculated with species of *Fusarium, C. scoparium, B. cinerea* and *R. solani* had significantly greater root disease scores those of uninoculated seedlings. Root disease scores of seedlings inoculated with *T. hamatum, T. harzianum, T. koningii, Bimuria novae zelandiae* and SHG 1 were not significantly ($P<0.05$) greater than those of uninoculated seedlings (Figure 3.52). These species along with *T. basicola, M. gamsii* and *D. dematioidea* also produced no appreciable root symptoms on grass seedlings (Figure 3.53). Results indicating that the *Trichoderma* species tested were not pathogenic to seed or seedlings contrasts with some of the results obtained using the axenic seedling Petri plate technique. This pot method may therefore be more reliable to determine the pathogenicity of *Trichoderma* isolates than the Petri plate method as results were more consistent. However, most other fungi were again shown to be pathogenic to germinated seedlings of legumes and grasses, confirming the results found with the previous Petri plate method.

The fungi reisolated from infected seed and seedling tissue were identified as those used to inoculate the seedlings thus satisfying Koch's postulates. As this test was not undertaken in sterile conditions, other fungi were sometimes isolated from infected seeds and seedlings, and were most likely secondary invaders.

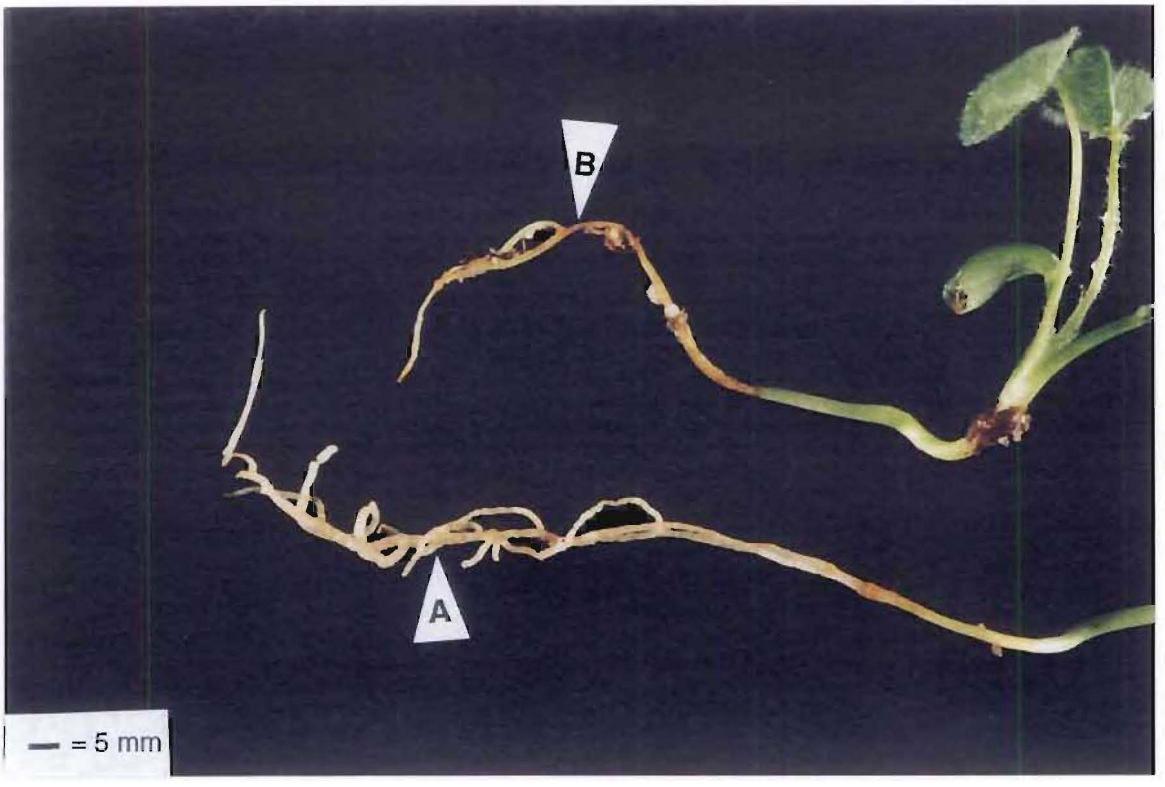


Figure 3.48 Subterranean clover seedlings showing healthy white roots of uninoculated control (A) and seedlings inoculated with *Botrytis cinerea* (B).

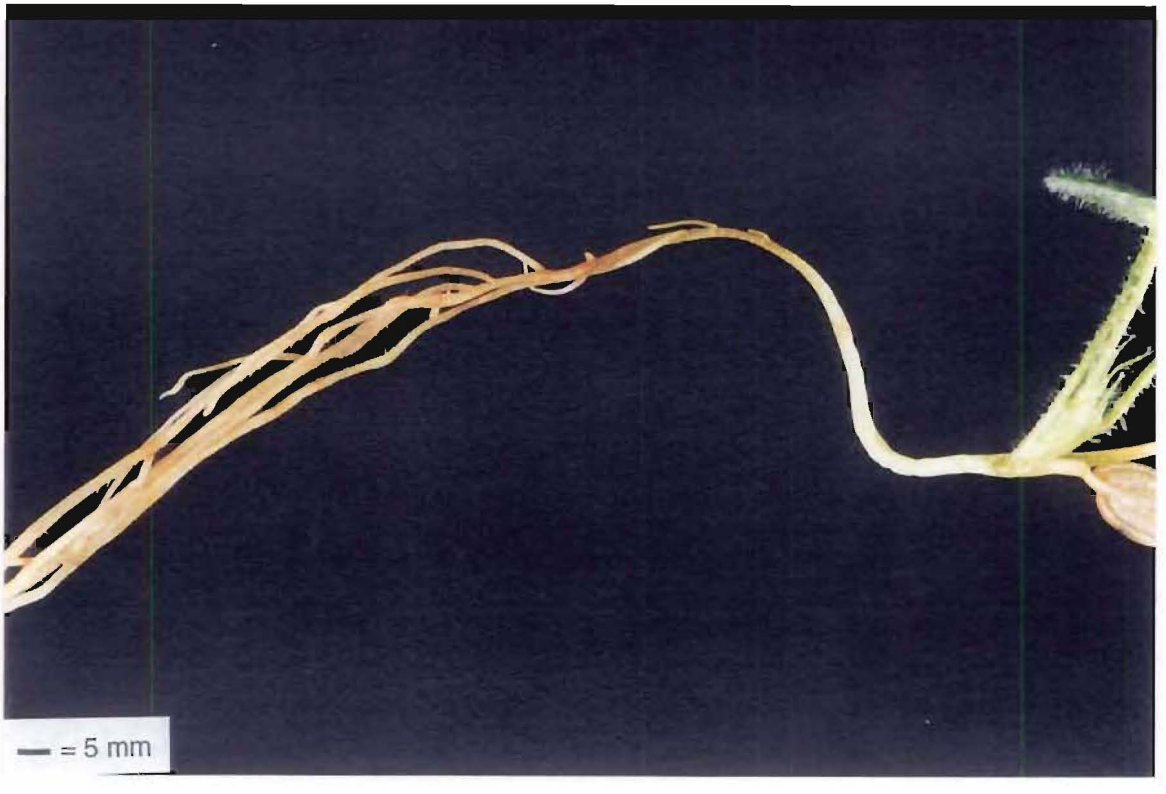


Figure 3.49 Brown discoloration on subterranean clover inoculated with *Rhizoctonia solani*

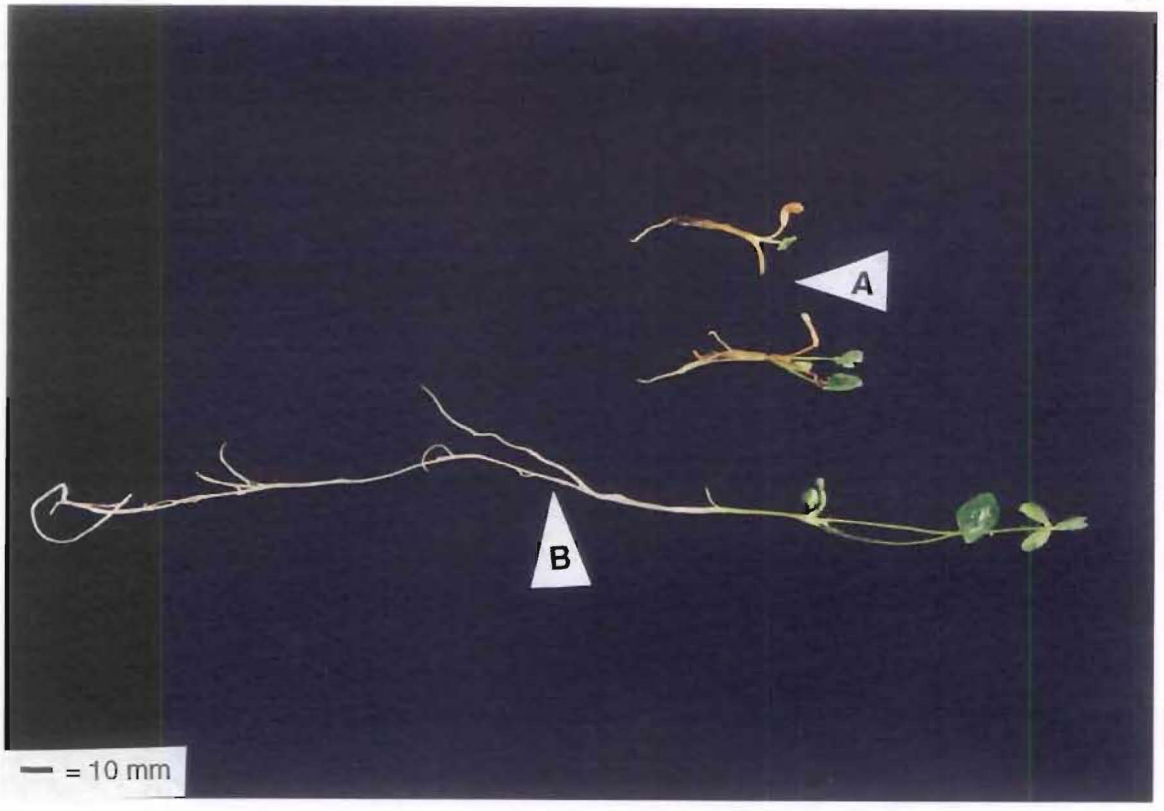


Figure 3.50 A comparison of root growth of subterranean clover (A) seedlings inoculated with *Fusarium crookwellense* and (B) uninoculated control seedlings.

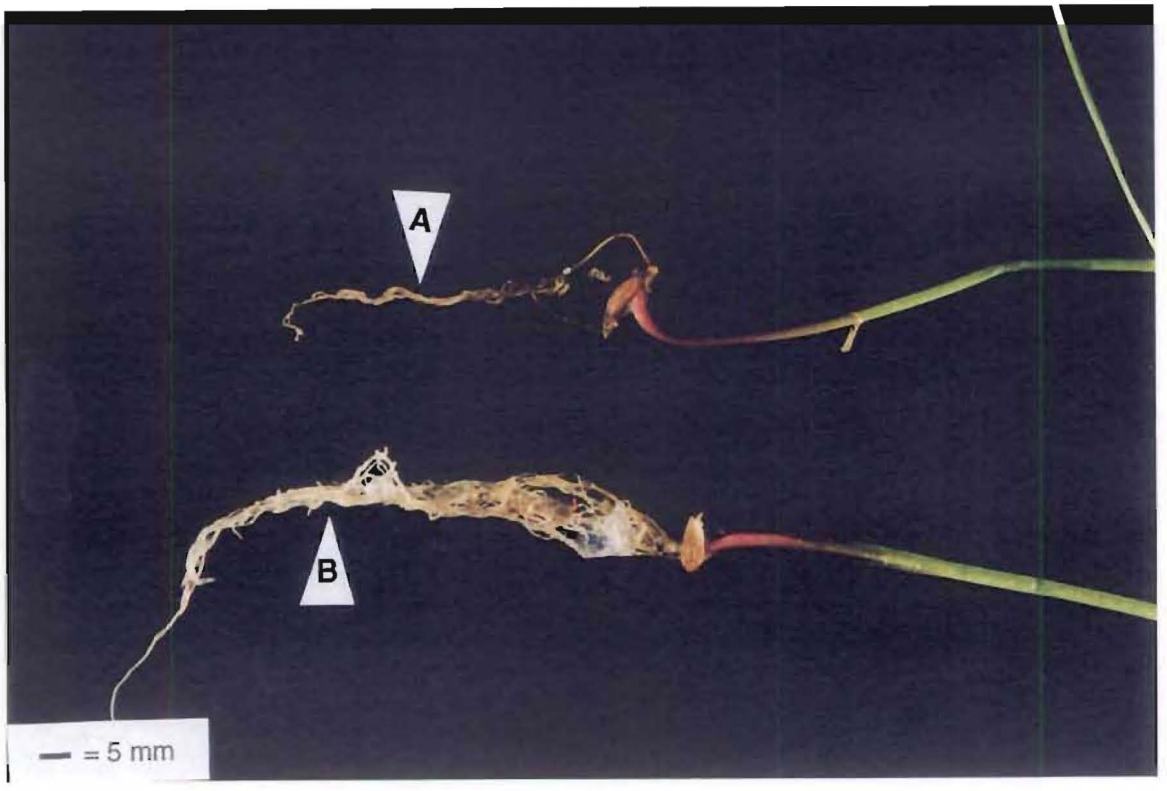
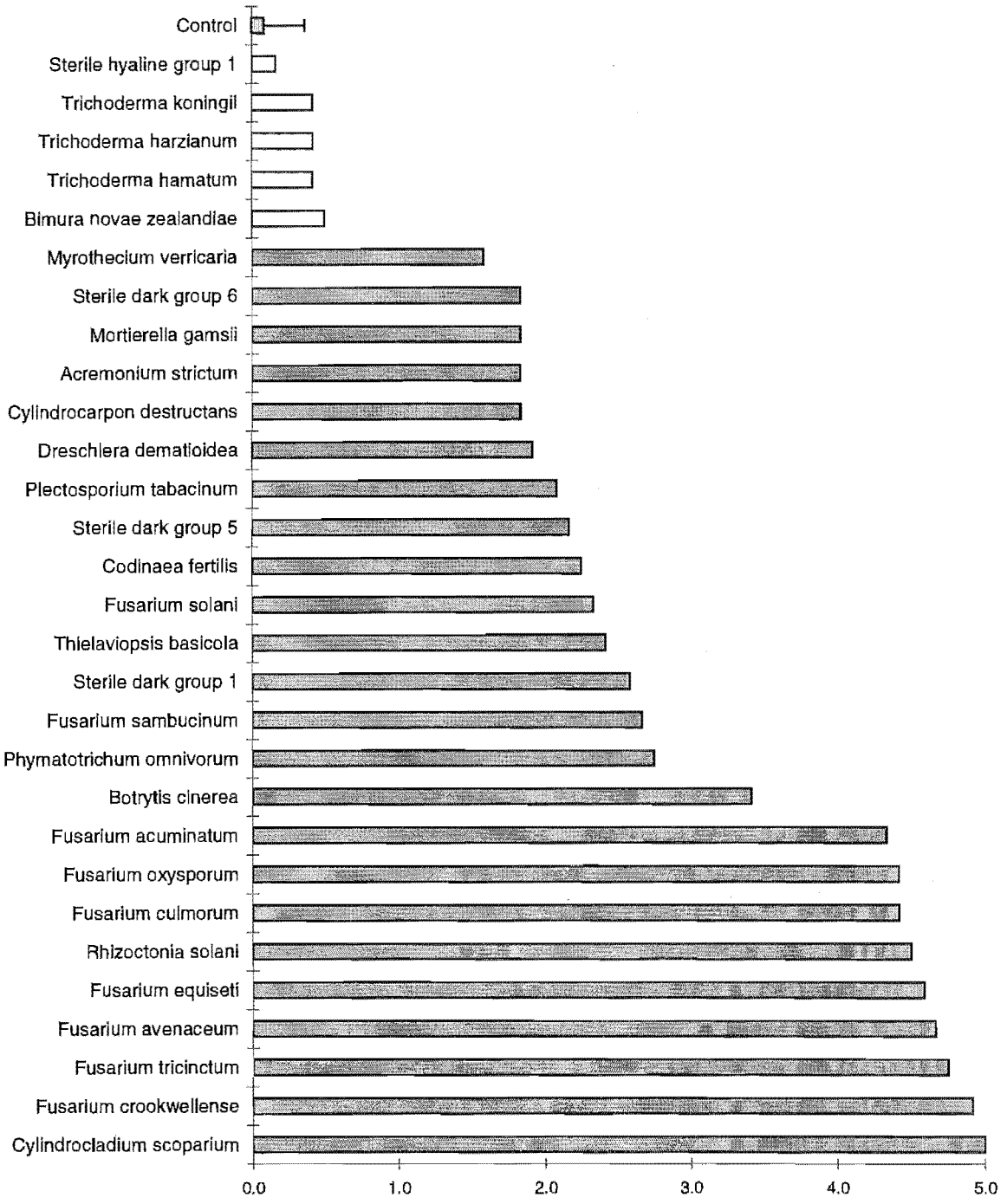
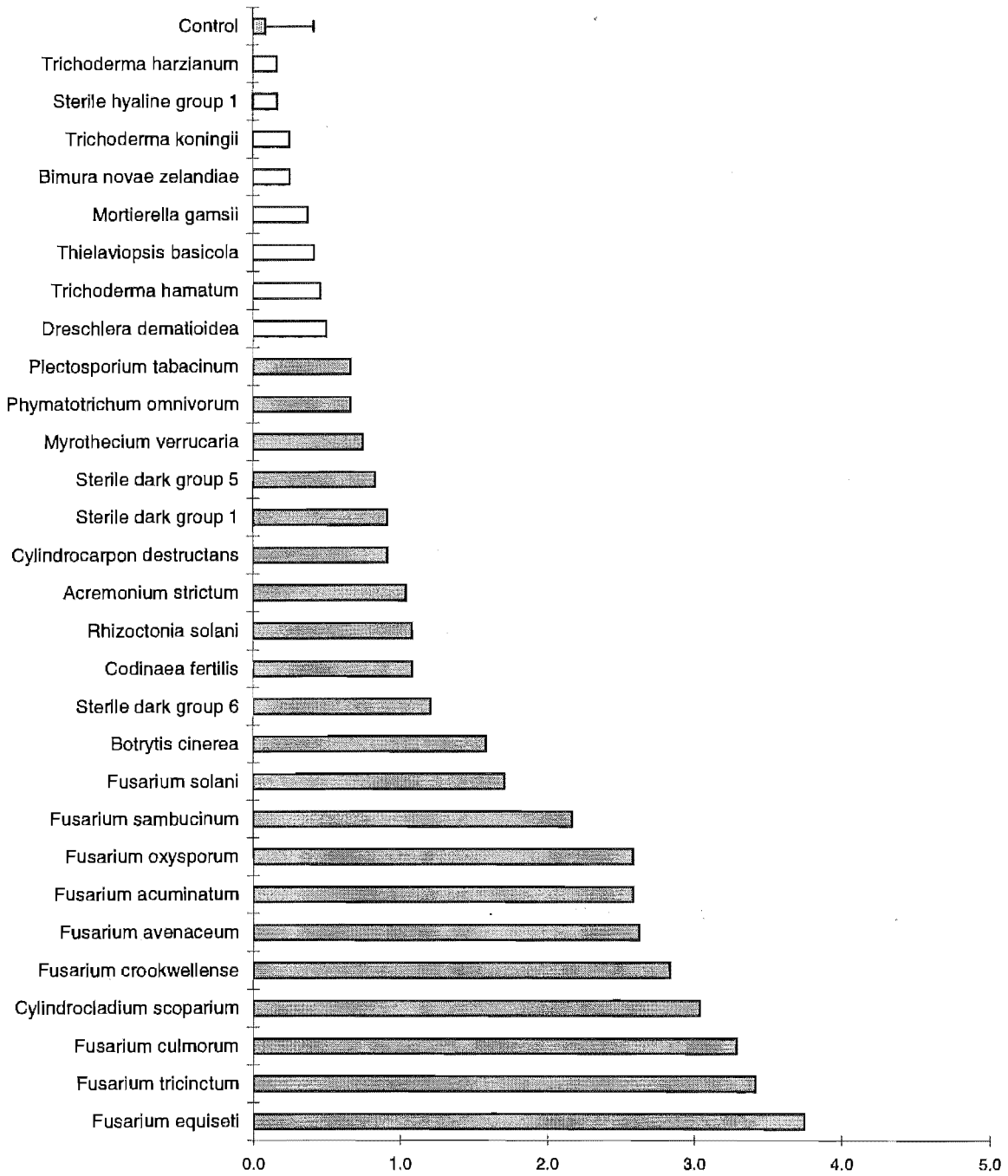


Figure 3.51 A comparison of root growth of ryegrass (A) seedlings inoculated with *Fusarium crookwellense* and (B) uninoculated control seedlings.



Error bar = SEM 0.282
Shaded bars indicate fungal treatments with significantly higher disease compared to the control P< 0.05

Figure 3.52 Mean root disease score of four legume hosts inoculated with pathogenic fungi.



Error bar = SEM 0.331
Shaded bars indicate fungal treatments with significantly higher root disease compared to the control P< 0.05

Figure 3.53 Mean root disease score of eight grass hosts inoculated with pathogenic fungi

Many of these soilborne root pathogens have the capacity to infect and kill seed and seedlings and can therefore be considered as both seed and plant root pathogens. The seedling emergence of legume plants was reduced following inoculation of a broad range of root- pathogenic fungi which contrasted with the low number of fungi which reduced emergence of grass seedlings. In particular, species of *Fusarium, Botrytis cinerea* and *Rhizoctonia solani* were highly pathogenic to seed of both grasses and legumes as reported elsewhere. *Fusarium avenaceum, F. oxysporum, F. equiseti, F. solani* and *F. acuminatum* have been isolated from seed of subterranean clover (McGee and Kellock 1974, Barbetti 1984a), red clover (Kovacikova and Kudela 1989, 1990) and white clover (Kellock *et al.* 1978) and *Fusarium culmorum* was a virulent seedborne root pathogen of grasses and cereals (Holmes 1979, 1983), a result repeated here. *Botrytis cinerea* and *R. solani* have also been reported to be seed pathogens of clovers (MacNish 1977, Mebalds 1988). This study found *F. crookwellense, F. sambucinum* and *F. tricinctum* to be virulent pasture seed pathogens, none of which have been previously reported as seed pathogens in similar studies. The infection of seed and seedlings by fungal pathogens reduced emergence and affected the establishment of plants in pasture swards. The emergence of forage legumes was adversely affected by fungal pathogens compared to their companion grass species. This may be a contributing factor to the botanical composition and clover decline of Waikato pastures.

Falloon (1985) reported that *Penicillium* spp., *Aspergillus* spp., *Curvularia trifolii* and *Chaetomium globosum* were seedborne pathogens of New Zealand ryegrass cultivars. These fungi were also isolated from pasture roots in this study but were mostly non-pathogenic to axenically grown seedlings. This indicates that some fungi which are specialised seedborne pathogens are non-pathogenic to plant roots, for example *Fusarium equiseti* was highly pathogenic to seeds of all legume and grass seeds but was only weakly pathogenic to all grass seedlings. Further work with non-pathogenic root fungi isolated from Waikato pastures is required to elucidate the full spectrum of fungi present in pasture which may reduce seed emergence.

3.3.3 EFFECT OF ROOT FUNGI ON THE YIELD OF SHOOT AND ROOTS OF PASTURE PLANTS

3.3.3. (a) Inoculation of plants grown in fumigated soil

All plants inoculated with non-pathogenic fungi had similar dry weights to control plants, excluding plants inoculated with *P. carneus* and *T. tocklaiensis* where the mean dry shoot weights were lower than the controls (Table 3.15). Plants inoculated with pathogenic fungi showed a varied response. Some hosts had significantly lower mean dry shoot weights to than controls following inoculation with some pathogens, but not others. The only exception to this was endophyte free ryegrass where all pathogens, except *F. crookwellense*, caused a significant decrease in shoot weight. All plants inoculated with *F. tricinctum* and *C. scoparium* had lower shoot weights than controls (Figures 3.54 - 3.56). Results generally indicated that most fungal treatments had no effect on the mean dry shoot weight of most hosts.

Table 3.15 Mean dry shoot weight (g) of plants grown in fumigated soil and inoculated with pathogenic and non-pathogenic root- colonising fungi.

Fungal species	Ryegrass 1	Ryegrass 2	White clover	Sweet vernal	Browntop	Soft brome
PATHOGENS						
Fusarium acuminatum	0.543*	0.577	0.183*	0.343*	0.527	0.547
Fusarium avenaceum	0.720+	0.243*	0.320	0.453	0.407*	0.390*
Fusarium crookwellense	0.393*	0.447*	0.267	0.410	0.233*	0.400*
Fusarium culmorum	0.433*	0.537	0.317	0.307*	0.570	0.450
Fusarium equiseti	0.510*	0.450*	0.253	0.317*	0.480	0.533
Fusarium oxysporum	0.507*	0.550	0.250	0.440	0.457*	0.363*
Fusarium sambucinum	0.453*	0.457*	0.240*	0.390	0.580	0.560
Fusarium solani	0.440*	0.420*	0.327	0.480	0.400*	0.470
Fusarium tricinctum	0.223*	0.363*	0.210*	0.317*	0.417*	0.163*
Cylindrocladium scoparium	0.467*	0.573	0.013*	0.073*	0.393*	0.343*
Cylindrocarpon destructans	0.420*	0.603	0.310	0.370	0.463*	0.500
Codinaea fertilis	0.477*	0.640	0.310	0.420	0.577	0.540
Myrothecium verrucaria	0.360*	0.680	0.330	0.377	0.587	0.507
NON PATHOGENS						
Gongronella butleri	0.573	0.537	0.333	0.417	0.507	0.513
Mariannaea elegans	0.657	0.637	0.373+	0.550+	0.630+	0.623+
Paecilomyces carneus	0.427*	0.447*	0.356	0.400	0.577	0.467
Thozetella tocklaiensis	0.540*	0.530	0.356	0.387	0.533	0.473
Verticillium chlamydosporium	0.640	0.570	0.300	0.393	0.607	0.433
CONTROL (A)+(B)	0.655	0.628	0.310	0.410	0.540	0.480
SED	**0.105**	**0.101**	**0.060**	**0.074**	**0.076**	**0.064**

1 = Ryegrass endophyte free Nui, 2 = Ryegrass endophyte infected Nui.
*** denotes a significant difference (P< 0.05) from the control,**
+ denotes a significant increase in weight compared to the controls (P<0.05)

Results indicated that most pathogenic fungi reduced mean dry root weight on a majority of hosts (Table 3.16). White clover root weight was reduced by more fungal treatments than other the hosts, while reduced root weight of the grass hosts varied between

fungal treatments. In contrast to the shoot weight results, only five fungal treatments caused reduced root weights of endophyte free perennial ryegrass. All pathogens caused reduced root weight in at least one or more host, excluding *F. oxysporum* which did not reduce dry weight and *C. fertilis* which only affected white clover root weight. *Cylindrocladium scoparium* reduced the root weight of all inoculated plants and *C. destructans, F. avenaceum, F. crookwellense, F. culmorum,* and *F. tricinctum* also reduced root weights of most plants. *Verticillium chlamydosporium* was the only non-pathogenic fungal treatment to significantly lower plant root weight on any host.

Excluding ryegrass, all plants that were inoculated with the non-pathogenic fungus *Mariannaea elegans* had a significant increase in their mean dry shoot weights (Table 3.15) which may suggest this fungus has a beneficial or stimulatory effect on shoot growth, This result was not repeated for mean dry root weights (Table 3.16) as only sweet vernal and endophyte free ryegrass had increased root weights. Browntop plants inoculated with *G. butleri, T. tocklaiensis* and *V. chlamydosporium* also had higher dry root weights than the control plants.

Table 3.16 Mean dry root weight (g) of plants grown in fumigated soil and inoculated with pathogenic and non-pathogenic root-colonising fungi.

Fungal species	Ryegrass 1	Ryegrass 2	White clover	Sweet vernal	Browntop	Soft brome
PATHOGENS						
Fusarium acuminatum	0.327*	0.373	0.177*	0.170*	0.240*	0.480
Fusarium avenaceum	0.583+	0.183*	0.107*	0.183*	0.220*	0.183*
Fusarium crookwellense	0.293*	0.127*	0.127*	0.197*	0.107*	0.470
Fusarium culmorum	0.430	0.407	0.120*	0.123*	0.183*	0.330*
Fusarium equiseti	0.340	0.287*	0.073*	0.227	0.203*	0.400
Fusarium oxysporum	0.460	0.427	0.207	0.280	0.420	0.383
Fusarium sambucinum	0.410	0.210*	0.103*	0.193*	0.367	0.327*
Fusarium solani	0.437	0.313*	0.150*	0.243	0.170*	0.510
Fusarium tricinctum	0.280*	0.220*	0.100*	0.160*	0.257*	0.150*
Cylindrocladium scoparium	0.303*	0.290*	0.010*	0.053*	0.073*	0.253*
Cylindrocarpon destructans	0.297*	0.437	0.110*	0.143*	0.180*	0.313*
Codinaea fertilis	0.390	0.517	0.107*	0.200	0.350	0.567
Myrothecium verrucaria	0.423	0.773	0.200	0.190*	0.270*	0.480
NON PATHOGENS	Ryegrass 1	Ryegrass 2	White clover	Sweet vernal	Browntop	Soft brome
Gongronella butleri	0.560	0.357	0.200	0.280	0.407+	0.390
Mariannaea elegans	0.933+	0.453	0.203	0.443+	0.317	0.500
Paecilomyces carneus	0.357	0.337	0.233	0.243	0.357	0.417
Thozetella tocklaiensis	0.433	0.353	0.190	0.310	0.627+	0.413
Verticillium chlamydosporium	0.457	0.420	0.213	0.180*	0.420+	0.483
CONTROL (A)	0.447	0.463	0.230	0.257	0.353	0.457
SED	**0.110**	**0.112**	**0.040**	**0.057**	**0.050**	**0.113**

1 = Ryegrass endophyte free Nui, 2 = Ryegrass endophyte infected Nui.
*** denotes a significant difference (P< 0.05) from the control**
+ denotes a significant increase in weight compared to the controls (P<0.05)

There was no consistent difference between the root yields of endophyte infected and endophyte free ryegrass plants. This may be in part due to the low levels of endophyte recorded in plant roots (di Menna pers. comm.). There was, however, a significantly higher shoot yield loss in non endophyte-infected plants than the infected plants. Further research would be required to explain and confirm this initial result.

Root discoloration and necrosis was more apparent on the roots of plants inoculated with pathogens than the controls and those plants inoculated with non pathogens (Table 3.17). Most pathogen treatments had higher disease scores than the controls, apart from *Fusarium acuminatum* where necrosis was absent on most roots.

Table 3.17 Mean root disease scores of plants grown in fumigated soil and inoculated with pathogenic and non-pathogenic root-colonising fungi.

Fungal species	Ryegrass 1	Ryegrass 2	White clover	Sweet vernal	Browntop	Soft brome
PATHOGENS						
Fusarium acuminatum	0.33	0.00	0.33	0.67	0.33	0.67
Fusarium avenaceum	1.00*	1.67*	1.33*	1.00*	1.33*	2.00*
Fusarium crookwellense	1.00*	1.33*	1.00*	1.67*	2.00*	0.67
Fusarium culmorum	3.00*	1.67*	1.33*	1.00*	2.00*	2.33*
Fusarium equiseti	1.00*	1.33*	1.33*	0.33	0.67	0.00
Fusarium oxysporum	1.00*	0.67	0.33	0.67	1.00*	0.33
Fusarium sambucinum	1.33*	1.00*	0.67	0.67	0.33	0.33
Fusarium solani	1.33*	0.33	0.67	0.67	0.67	0.67
Fusarium tricinctum	1.67*	1.33*	1.67*	1.33*	1.00*	2.67*
Cylindrocladium scoparium	2.00*	2.33*	4.67*	2.33*	1.67*	1.67*
Cylindrocarpon destructans	1.33*	1.33*	1.67*	0.67	1.00*	0.33
Codinaea fertilis	1.33*	0.00	1.67*	0.67	0.67	0.00
Myrothecium verrucaria	1.00*	0.00	1.00*	0.33	0.33	0.00
NON PATHOGENS						
Gongronella butleri	0.33	0.00	0.00	0.33	0.00	0.00
Mariannaea elegans	0.00	0.00	0.00	0.00	0.00	0.00
Paecilomyces carneus	0.00	0.00	0.00	0.00	0.00	0.00
Thozetella tocklaiensis	0.00	0.00	0.00	0.67	0.67	0.00
Verticillium chlamydosporium	0.00	0.33	0.33	0.33	0.33	0.00
CONTROL (A)	0.00	0.00	0.00	0.00	0.00	0.00
SED	**0.427**	**0.504**	**0.408**	**0.445**	**0.463**	**0.496**

1 = Ryegrass endophyte free Nui, 2 = Ryegrass endophyte infected Nui.
*** denotes a significant difference (P< 0.05) from the control**

3.3.3. (b) Inoculation of plants grown in non-fumigated soil

The mean dry shoot weight of ryegrass, clover and browntop was reduced by the inoculation of pathogens (Table 3.18, Figures 3.57- 3.59) and although results varied, all fungal treatments were able to affect at least one host. Sweet vernal shoot weights were unaffected by all fungal treatments.

Table 3.18 Mean dry shoot weight (g) of plants grown in non-fumigated soil and inoculated with pathogenic root-colonising fungi.

Fungal species	Ryegrass	White clover	Sweet vernal	Browntop
Fusarium avenaceum	0.18*	0.09*	0.26	0.15*
Fusarium crookwellense	0.19*	0.08*	0.24	0.17*
Fusarium culmorum	0.21*	0.08*	0.20	0.13*
Fusarium solani	0.32	0.08*	0.27	0.26
Fusarium tricinctum	0.18*	0.09*	0.22	0.24
Cylindrocladium scoparium	0.20*	0.01*	0.22	0.24
Cylindrocarpon destructans	0.23	0.10*	0.29	0.30
Codinaea fertilis	0.25	0.12	0.21	0.20*
CONTROL (A)	0.24	0.12	0.25	0.27
SED	**0.029**	**0.019**	**0.033**	**0.0316**

*** denotes a significant difference (P< 0.05) from the control**

Mean dry root weights of plants were also reduced by most fungal treatments (Table 3.19) except *Fusarium solani* which did not reduce any root weights. *Cylindrocladium scoparium, Fusarium crookwellense* and *Fusarium culmorum* reduced root weights of all inoculated plants and in contrast to the shoot results, these three fungi caused a significant reduction of sweet vernal root weight.

Table 3.19 Mean dry root weight (g) of plants grown in non-fumigated soil and inoculated with pathogenic root-colonising fungi.

Fungal species	Ryegrass	White clover	Sweet vernal	Browntop
Fusarium avenaceum	0.10*	0.04*	0.16	0.05*
Fusarium crookwellense	0.12*	0.03*	0.11*	0.08*
Fusarium culmorum	0.12*	0.007**	0.10*	0.02*
Fusarium solani	0.22	0.11	0.20	0.13
Fusarium tricinctum	0.12*	0.05*	0.16	0.07*
Cylindrocarpon destructans	0.24	0.05*	0.20	0.13
Cylindrocladium scoparium	0.10*	0.003**	0.10*	0.03*
Codinaea fertilis	0.16*	0.08*	0.17	0.10*
CONTROL (A)	0.23	0.11	0.15	0.12
SED	**0.036**	**0.021**	**0.03**	**0.015**

*** denotes a significant difference (P< 0.05) from the control, ** denotes a significant difference (P< 0.01) from the control.**

Root disease symptoms were observed on the majority of inoculated plants (Table 3.20), particularly on white clover inoculated with *Cylindrocladium scoparium* (Figure 3.60) and *Fusarium culmorum*, where severe root rot was recorded. *Fusarium solani* did not appear to be pathogenic to plants grown in non-fumigated soil as few symptoms were observed on roots and dry weights were not affected.

Table 3.20 Mean root disease score of plants grown in non-fumigated soil and inoculated with pathogenic root-colonising fungi.

Fungal species	Ryegrass	White clover	Sweet vernal	Browntop
Fusarium avenaceum	1.67*	1.33*	0.77*	0.67
Fusarium crookwellense	1.00*	1.50*	1.33*	1.50*
Fusarium culmorum	1.00*	3.33*	1.00*	2.50*
Fusarium solani	0.67	0	0	0
Fusarium tricinctum	1.00*	1.67*	1.00*	1.67*
Cylindrocarpon destructans	0.67	2.00*	0	0.17*
Cylindrocladium scoparium	1.33*	4.33**	1.00*	1.50*
Codinaea fertilis	0	1.00*	0.33	0
CONTROL (A)	0	0.33	0	0
SED	**0.371**	**0.360**	**0.392**	**0.398**

*** denotes a significant difference (P< 0.05) from the control, ** denotes a significant difference (P< 0.01) from the control.**

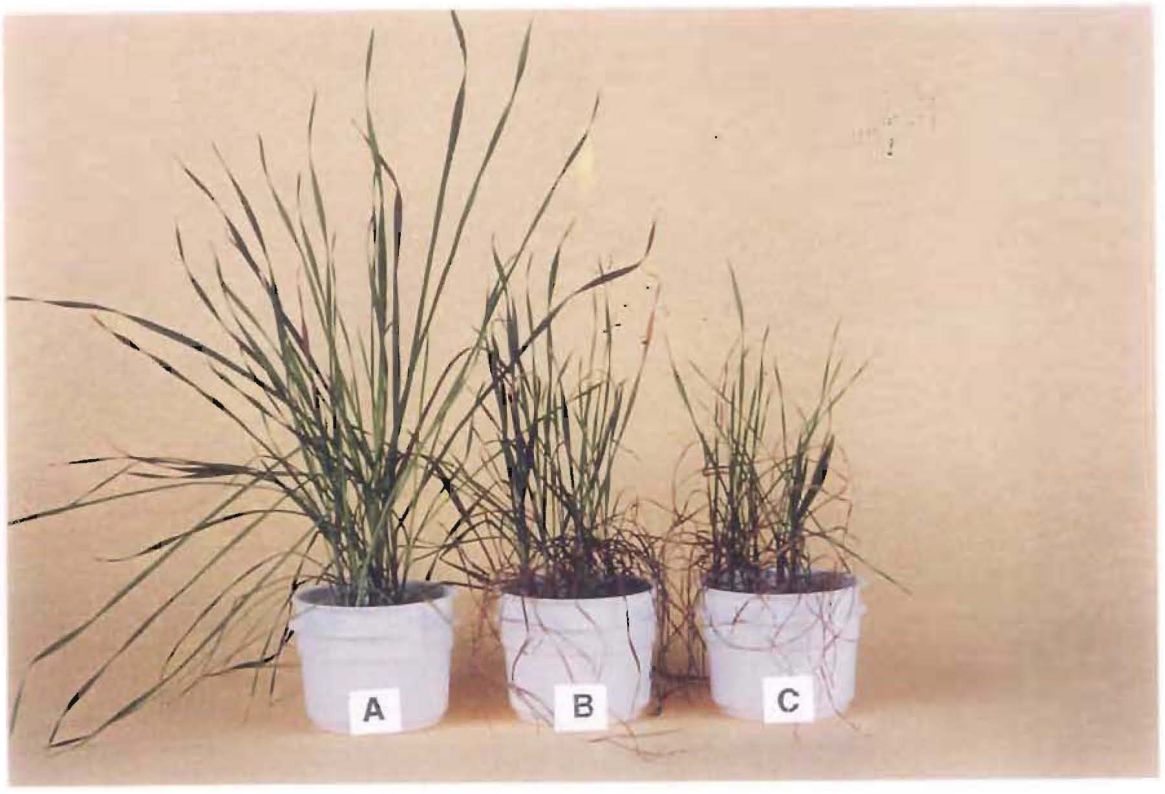


Figure 3.54 Soft brome plants grown in fumigated soil (A) uninoculated control, (B) inoculated with *Fusarium avenaceum*, (C) inoculated with *Fusarium tricinctum*.

Figure 3.55 Endophyte free perennial ryegrass grown in fumigated soil, uninoculated control (left), inoculated with *Fusarium tricinctum* (right).

Figure 3.56 Browntop grown in fumigated soil, uninoculated control (left), inoculated with *Cylindrocladium scoparium* (right).

Figure 3.57 White clover grown in non-fumigated soil, uninoculated control (left), inoculated with *Fusarium tricinctum* (right).

Figure 3.58 White clover grown in non-fumigated soil, uninoculated control (left), inoculated with *Cylindrocladium scoparium* (right).

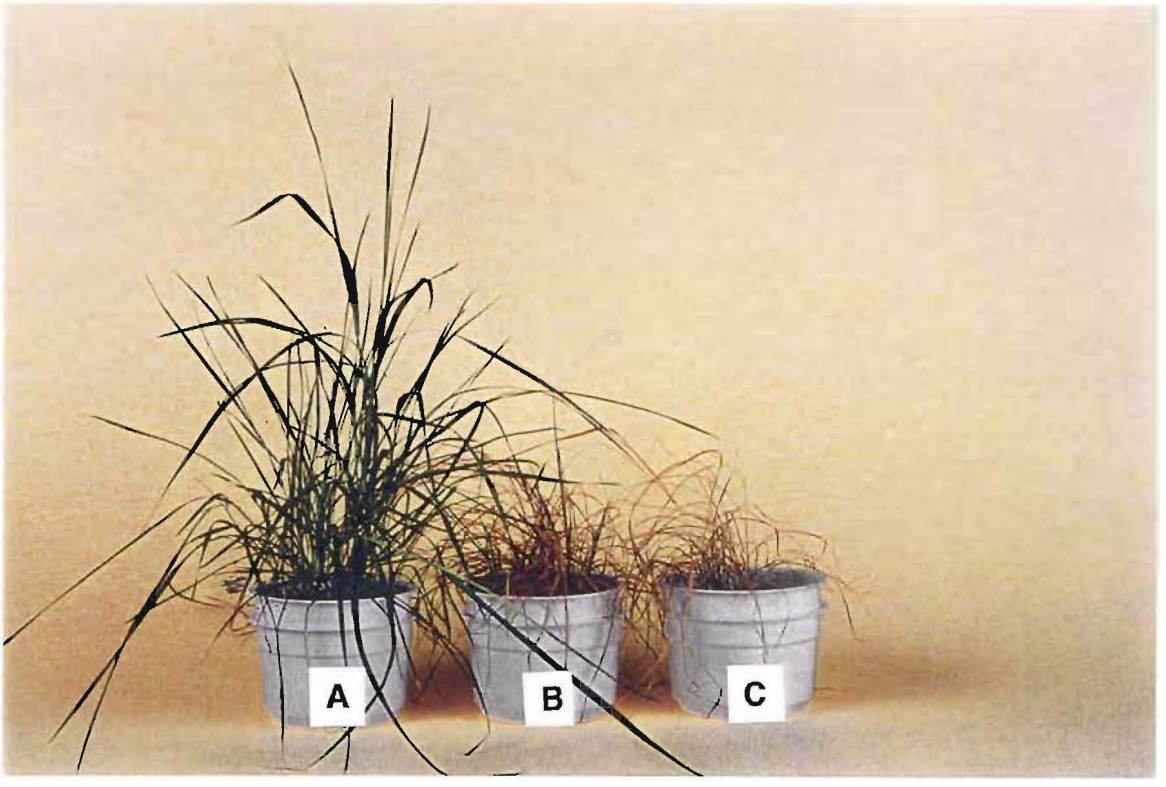


Figure 3.59 Browntop plants grown in non-fumigated soil (A) uninoculated control, (B) inoculated with *Fusarium crookwellense,* (C) inoculated with *Fusarium culmorum.*

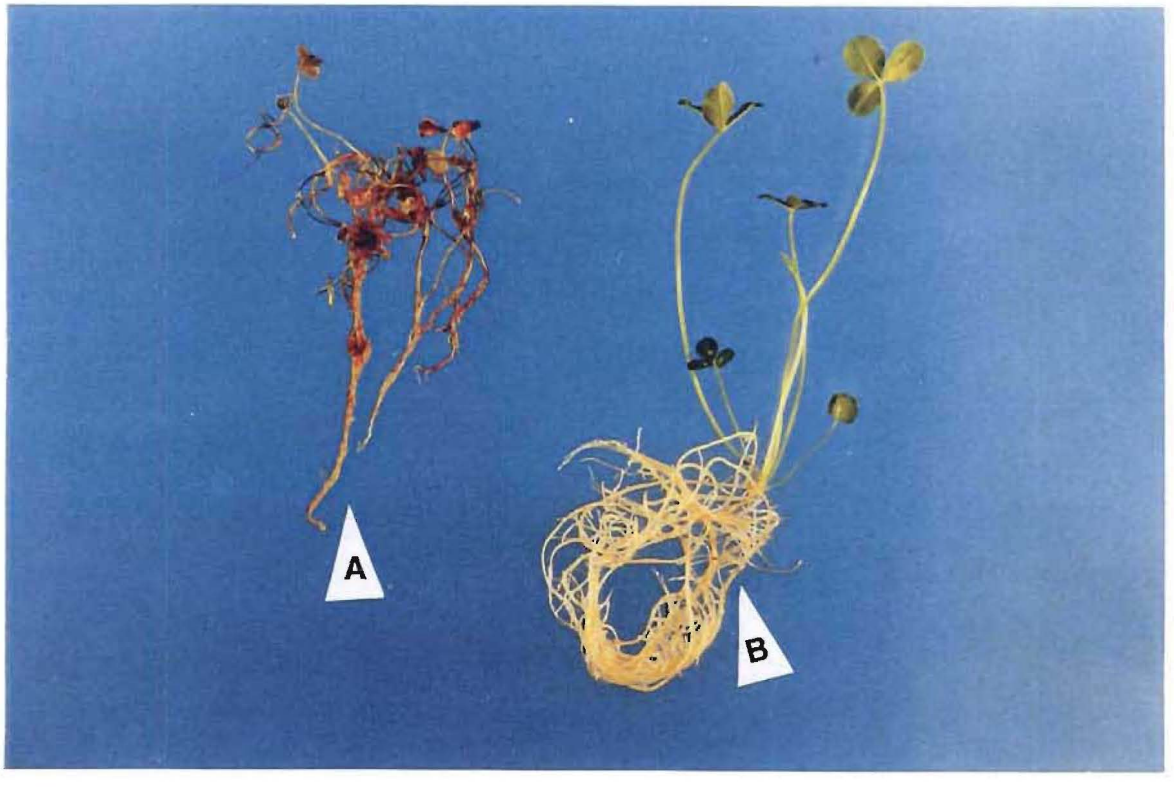


Figure 3.60 White clover plant grown in non-fumigated soil (A) diseased roots inoculated with *Cylindrocladium scoparium,* (B) uninoculated control

These results indicate that some soilborne root pathogens have the potential to reduce shoot and root yields of pasture plants. Pathogens, such as *Cylindrocladium scoparium,* can cause a severe loss of plant productivity (Freter and Wilcoxson 1964, Waipara *et al.* 1996c, Appendix 9), while the effect of the other pathogens on plant yields varied and was dependent on the host plant inoculated. White clover was more susceptible to yield losses than the grass species tested which again has implications for this species persistence and production in perennial pastures.

Root and shoot weights were reduced and disease symptoms increased in the presence of root pathogens in both sterilised and non sterilised soil, indicating these pathogenic fungi caused a loss of plant production independent from other pests and disease complexes. This is important as some studies have suggested fungi are opportunistic and secondary pasture pathogens rather than primary pathogens. Further research is required to investigate the impact of these individual pathogens in association with other organisms (both pathogens and non pathogens), as well as interactions with various plant stress factors. Studies on strain variation within a pathogen population will also help clarify production losses caused by pathogenic fungi.

Some non-pathogenic fungi, particularly *Mariannaea elegans*, caused an increase in plant yields which may indicate some of these fungi could stimulate plant growth, however results were inconsistent and require further investigation to be conclusive.

The results of this study were obtained in conditions that are unlike those that occur naturally and demonstrate only pathogenic potential which could be influenced by other factors under field conditions. Therefore future field assessment of pathogenicity to these hosts is a critical area for pasture root pathology.

3.3.4 EFFECT OF MOISTURE AND TEMPERATURE ON PATHOGENICITY OF ROOT PATHOGENS TO WHITE CLOVER.

3.3.4.1 *Codinaea fertilis*

Codinaea fertilis was reisolated from discolored and necrotic root segments of inoculated plants from all soil moisture treatments. Mean shoot and root dry weights of inoculated plants were all lower ($P<0.01$) than those of control plants at all soil moisture contents except at 80% WHC, where reduced shoot and root weights of strain #2 were not significantly lower than the controls (Figures 3.61, 3.62). Mean shoot dry weights also decreased as soil moisture decreased (Figure 3.61), with both control and inoculated plants grown in soil of 40% WHC again having the lowest shoot yields. Mean root dry weights in all three treatments decreased as the soil moisture content decreased (Figure 3.62), this

affect being most noticeable at 40% and 50% WHC where the yield of inoculated plants was half that of the controls.

Strain #1 of *C. fertilis* was more pathogenic to white clover than strain #2 particularly at higher soil moisture contents. Mean dry weight yields of plants inoculated with strain #1 were lower ($P < 0.01$) at 80% WHC than those of plants from strain #2 and the controls (Figures 3.61 and 3.62). Soil moisture affected the pathogenicity of *C. fertilis*, where plants not under water stress were less susceptible to yield loss and root damage than water-stressed plants. The difference in root and shoot yield reduction caused by the two strains indicates that the fungus has variable pathogenicity to white clover.

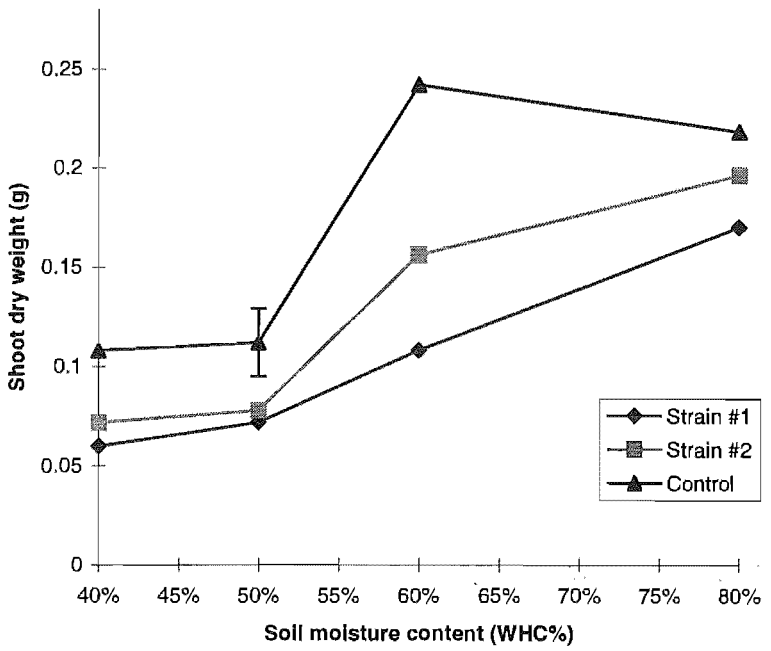


Figure 3.61 Mean shoot dry weight of white clover plants inoculated with *C. fertilis* and maintained at four levels of soil moisture. Error bar = SED, 0.021.

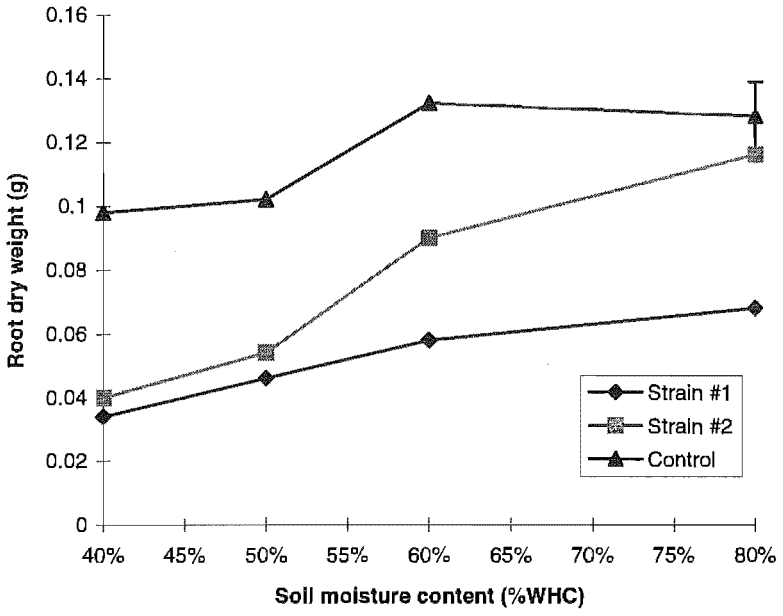


Figure 3.62 Mean root dry weight of white clover plants inoculated with *C. fertilis* and maintained at four levels of soil moisture. Error bar = SED, 0.034.

Roots of inoculated plants had a high incidence of conspicuous disease symptoms such as discoloration, lesions and root rot as shown in Figure 3.63, where inoculated plants had much higher disease scores than control plants. Roots were therefore more susceptible to damage and rot at low soil moisture contents.

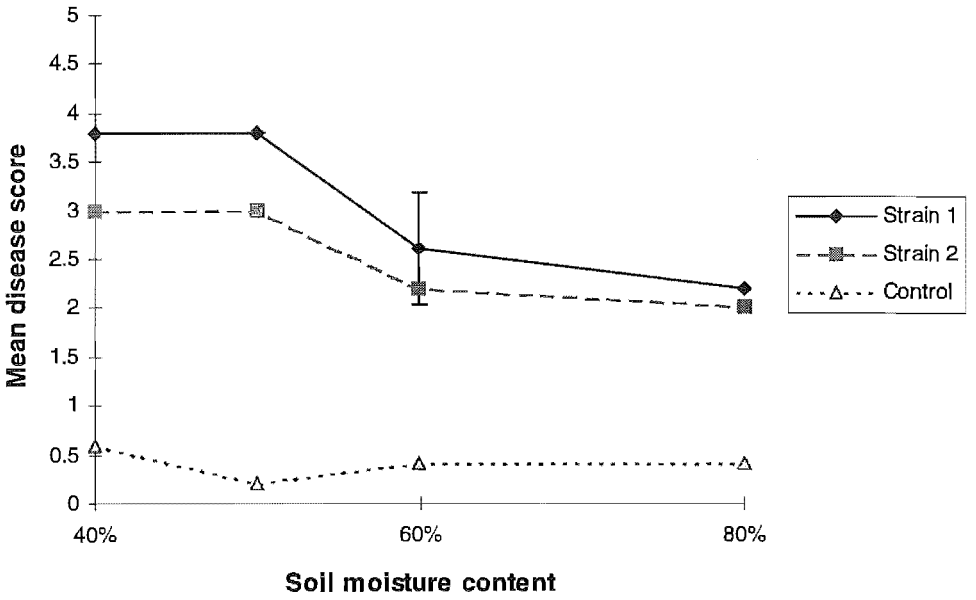
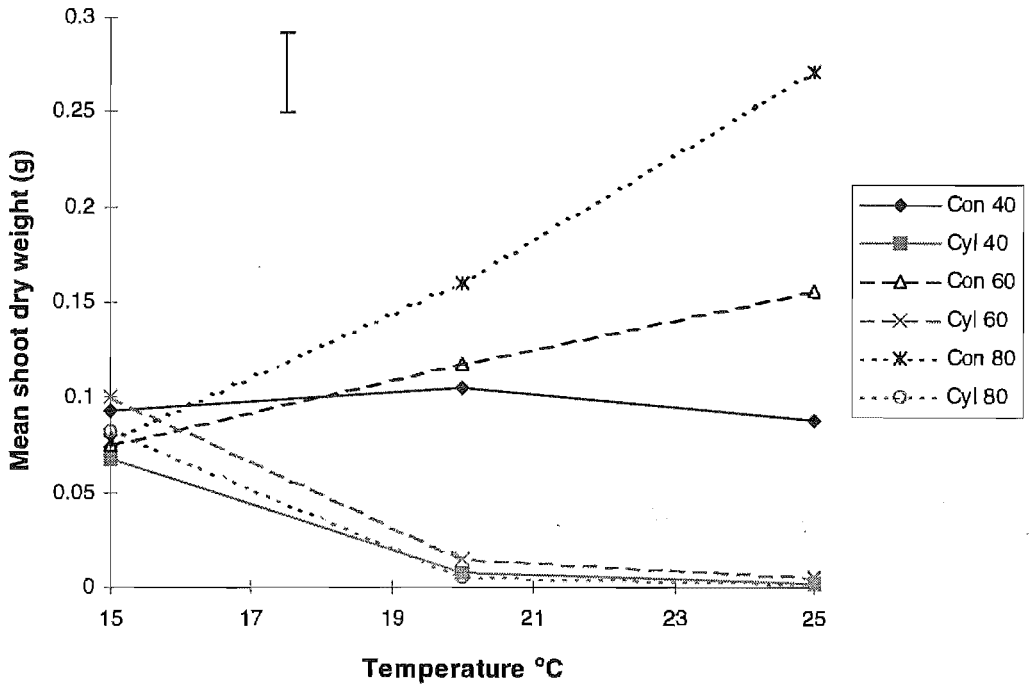


Figure 3.63 Mean root disease score of white clover plants inoculated with *C. fertilis* and maintained at different levels of soil moisture. Error bar = SED, 1.16

Disease symptoms of *C. fertilis* infection have previously been reported by Menzies (1973a) and Campbell (1982). *Codinaea fertilis* is a root-invading hyphomycete fungus (Appendix 3), and has been reported to cause substantial root rotting of many forage legume species (Campbell 1980, Campbell 1982, Skipp and Christensen 1982, Waipara *et al.* 1996b, Waipara *et al.* 1996a). This fungus has frequently been isolated from roots of white clover (Skipp and Christensen 1983) and perennial ryegrass (Skipp and Christensen 1989a) in warm areas of New Zealand (North Island and north west of the South Island), and comprised almost 10% of all isolates obtained from plant species in this study. Menzies (1973a) suggested that the widespread disappearance of white clover from some pastures, particularly in drought prone eastern areas of the North Island, may have been caused by *C. fertilis* root rot. Clover plants growing in the field could be susceptible to the same damage that was demonstrated in this study as low soil moisture contents (between 32-50% WHC) have also been measured in Waikato pasture soils during summer.

3.3.4.2 *Cylindrocladium scoparium*

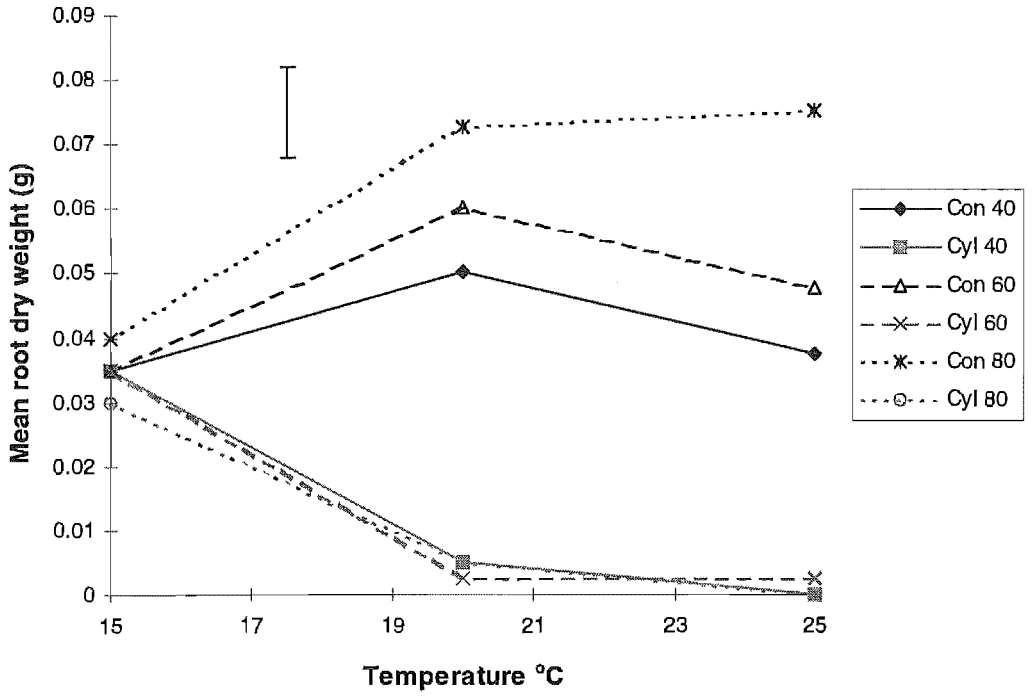
There were significant differences between the dry weight yields of white clover plants inoculated with *C. scoparium* and the uninoculated control plants. There was also a significant interaction between temperature, soil moisture and fungal inoculation. The mean shoot dry weight of inoculated plants was lower at all soil moisture levels at both 20°C and 25°C compared to control plants (Figure 3.64) and shows soil moisture alone did not affect the yield of shoots. Temperature affected shoot weights of all treatments as inoculated plants had reduced weights compared to controls at both 20°C and 25°C. However, at 15°C there was no difference between inoculated and uninoculated plants.



Con = uninoculated control treatments, Cyl = *Cylindrocladium scoparium* treatments

Figure 3.64 Mean shoot dry weight (g) of white clover plants inoculated with *Cylindrocladium scoparium* and maintained at three moisture levels (40%, 60%, 80% WHC) and three temperatures. Error bar = SED, 0.042

A similar mean dry weight response was found for root yields (Figure 3.65). Root weights of inoculated plants were reduced at all soil moisture levels and temperatures, excluding the 15°C treatment where there was no significant difference between any treatment.

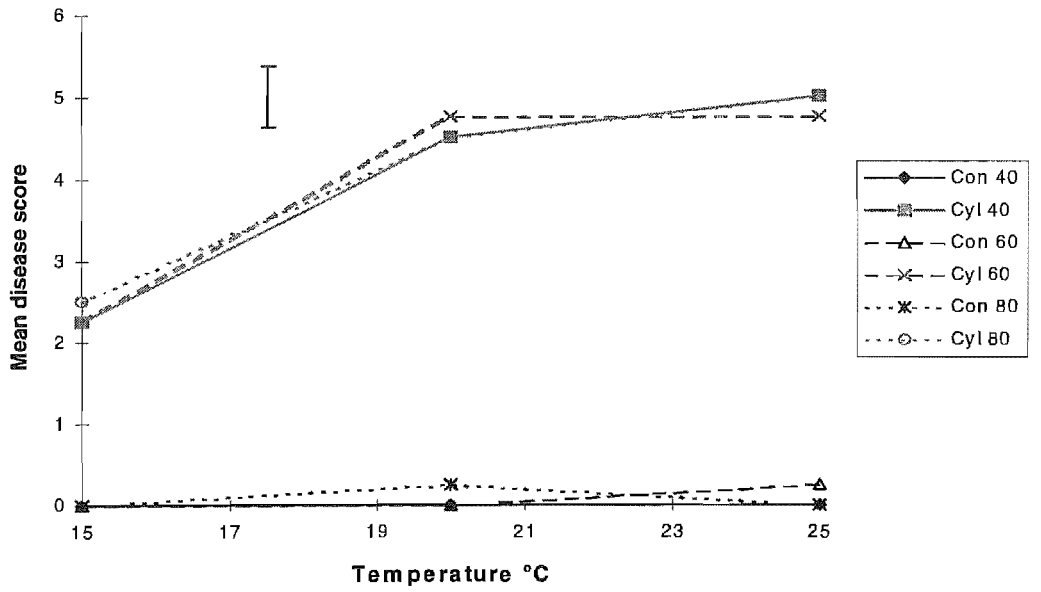


Con = control treatments, Cyl = *Cylindrocladium scoparium* treatments

Figure 3.65 Mean root dry weight (g) of white clover plants inoculated with *Cylindrocladium scoparium* and maintained at three moisture levels (40%, 60%, 80% WHC) and three temperatures. Error bar = SED, 0.014

There was a higher incidence of root disease symptoms on inoculated plants (Figure 3.66), with severe discoloration, rot and lesions being observed, and control roots remained white, turgid and unaffected. There was also less disease observed on inoculated plants at 15°C. The pathogen was reisolated from all inoculated root segments and was absent from the controls.

In general the yield of plants inoculated with *C. scoparium* was reduced most under conditions of high soil moisture and high temperature. The effect of this pathogen on shoot production is clearly illustrated in Figures 3.67 - 3.68.



Con = uninoculated control treatments, Cyl = *Cylindrocladium scoparium* treatments

Figure 3.66 Mean root disease score of white clover plants inoculated with *Cylindrocladium scoparium* and maintained at three moisture levels (40%, 60%, 80% WHC) and three temperatures. Error bar = SED, 0.77

Results showed that root and shoot weights were considerably reduced in the presence of this pathogen particularly at higher temperatures. This fungus has been implicated in economically important diseases and is reported to incite serious root rot in over 30 families of plants throughout the world (Freter and Wilcoxson 1964, Thies and Patton 1970, Hunter 1992). In previous pasture pathology studies *C. scoparium* has been isolated from red clover, sweet clover, white clover and lucerne plants (Appendix 3) (Ponappa *et al.* 1977, Willis 1965, Skipp *et al.* 1986, Skipp and Christensen 1990, Sarathchandra *et al.* 1995), and was also found to significantly damage and kill plants in pot trials (Skipp *et al.* 1986, Nan *et al.* 1991b). In this study, *C. scoparium* was isolated from surface sterilized roots of subterranean clover and perennial ryegrass and was present in 3% of white clover roots sampled (Waipara *et al.* 1996c). These isolation and pathogenicity results demonstrate that this pathogen could have a significant effect on white clover health and persistence in Waikato pastures particularly in early summer when temperatures and soil moisture would be suitable for this fungus to cause extensive root rot damage. However, mean annual soil temperatures in Waikato soils are closer to 15°C (Pengelly pers. comm.) which would reduce or suppress the activity of this pathogen for most of the year.

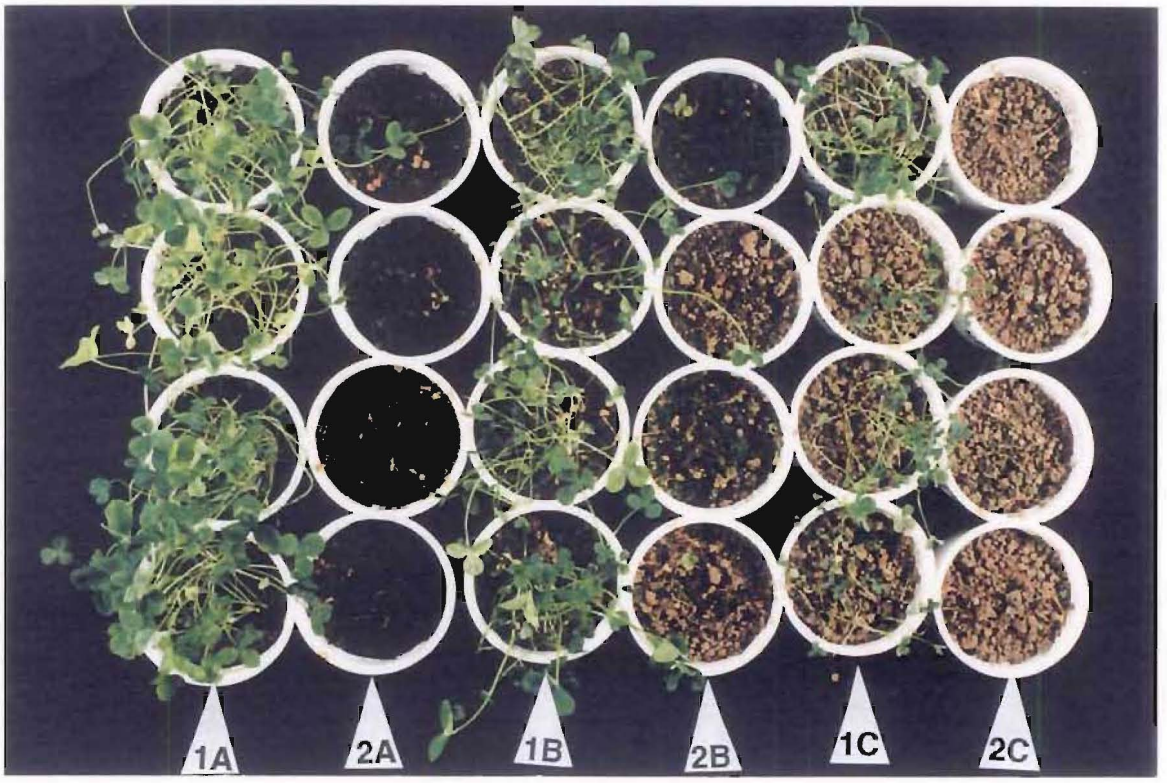


Figure 3.67 Uninoculated white clover plants (1) and plants inoculated with *Cylindrocladium scoparium* (2) at 25°C and maintained at (A) 80% WHC, (B) 60% WHC, (C) 40% WHC, 4 weeks after inoculation.

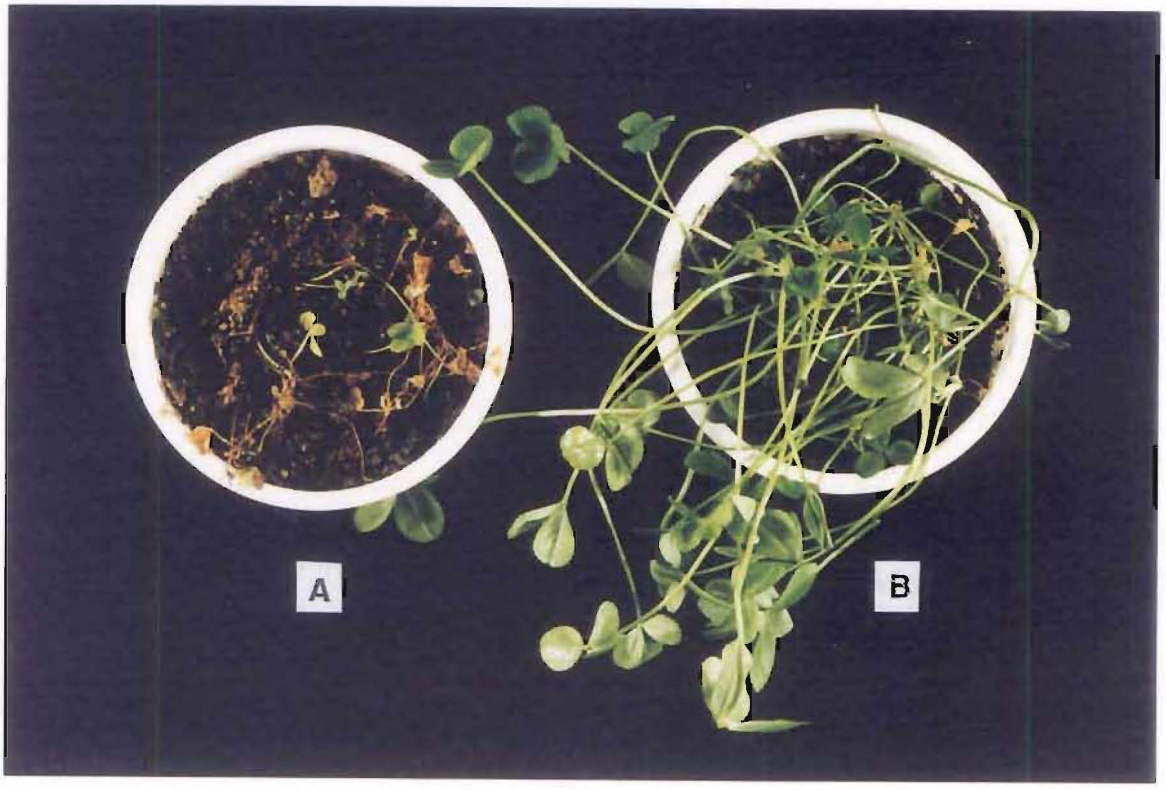


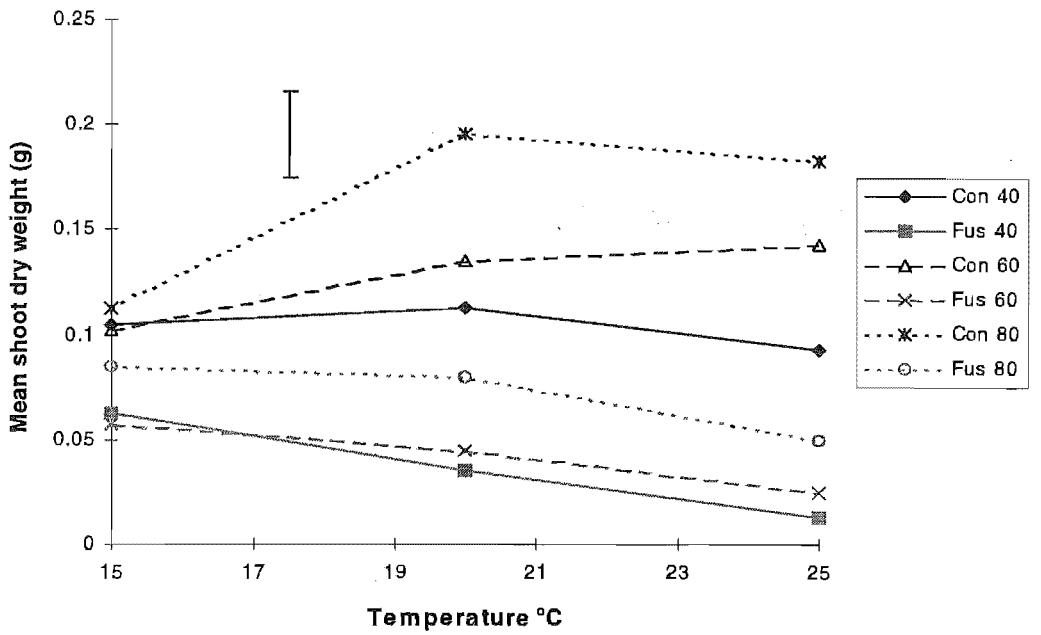
Figure 3.68 White clover plants inoculated with *Cylindrocladium scoparium* (A) and uninoculated control plants (B) at 25°C and maintained at 80% WHC, 4 weeks after inoculation.

3.3.4.3 *Fusarium crookwellense*

There were significant differences between the dry weight yields of white clover plants inoculated with *Fusarium crookwellense* and the uninoculated control plants. The mean shoot and root dry weights of inoculated plants was reduced at 60% and 80 % WHC at both 20° and 25°C (Figures 3.69, 3.70). However, there was no difference in either shoot or root dry weights between all treatments at 15°C, or all treatments at 40% WHC.

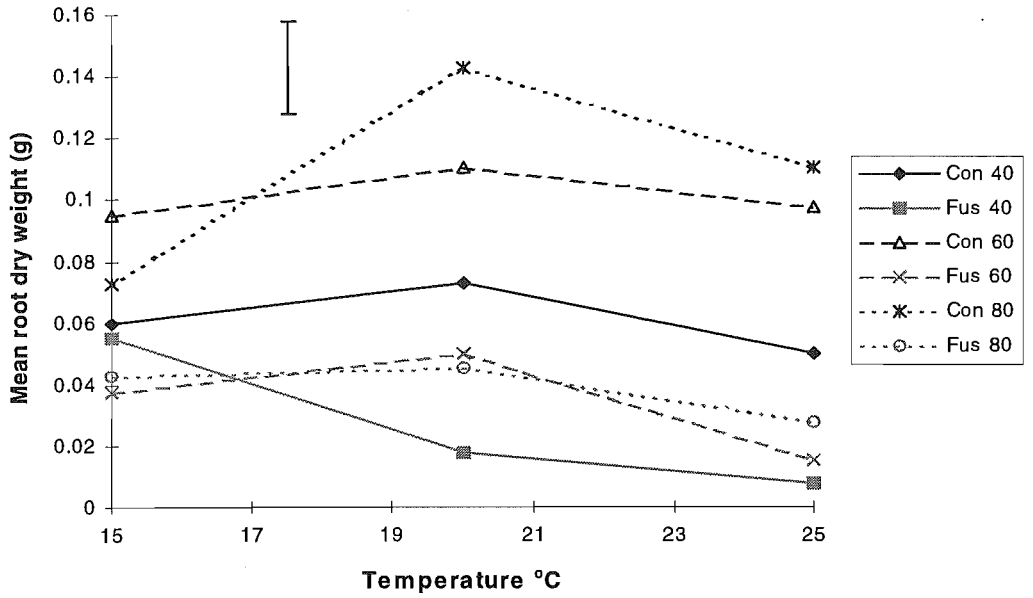
Control shoot weights of plants maintained at 60% and 80% WHC at both 20°C and 25°C were higher than all inoculated treatments. Control plants maintained at 40% WHC and inoculated plants maintained at 80% WHC had similar shoot and root weights at all temperatures.

All plant roots inoculated with *Fusarium crookwellense* had a high incidence of root rot and necrosis compared to the control plants (Figure 3.71) and disease severity was particularly higher at the low soil moisture (40% WHC) and high temperature (25°C) treatments



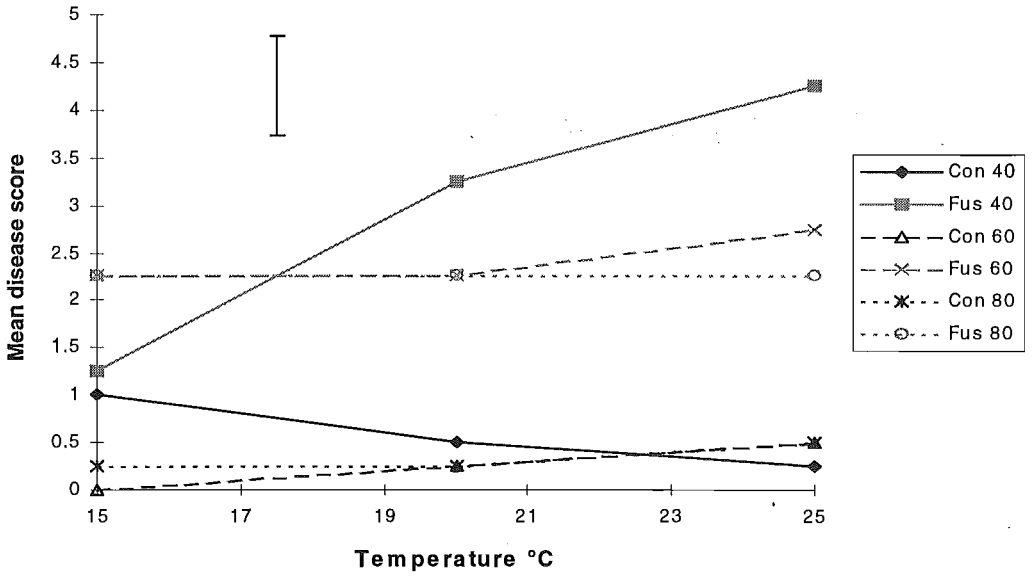
Con = uninoculated control treatments, Fus = *Fusarium crookwellense* treatments

Figure 3.69 Mean shoot dry weight (g) of white clover plants inoculated with *Fusarium crookwellense* and maintained at three moisture levels and three temperatures. Error bar = SED, 0.041



Con = uninoculated control treatments, Fus = *Fusarium crookwellense* treatments

Figure 3.70 Mean root dry weight (g) of white clover plants inoculated with *Fusarium crookwellense* and maintained at three moisture levels and three temperatures. Error bar = SED, 0.03



Con = uninoculated control treatments, Fus = *Fusarium crookwellense* treatments

Figure 3.71 Mean root disease score of white clover plants inoculated with *Fusarium crookwellense* and maintained at three moisture levels and three temperatures. Error bar = SED, 1.03

These results showed the pathogenicity of *F. crookwellense* was affected by both temperature and soil moisture. Plant yields and disease severity indicated that this fungus was more pathogenic at low soil moisture levels and high temperatures. It was also less

pathogenic at high soil moisture levels, which is in contrast to the low yield results obtained high moisture levels after inoculation of *C. scoparium*. Abiotic factors in conjunction with soilborne microbes must therefore be considered when the pathogenicity of root pathogens is measured.

There have been no previous reports of this fungus (or under its European synonym *Fusarium culmorum* var. *cerealis*) causing serious root damage to any plant hosts in the field. Until now *F. crookwellense* has been regarded as a weak or secondary root pathogen (Burgess *et al.* 1988). These results show this fungus to be a pathogen of white clover and because *F. crookwellense* was the second most frequently isolated *Fusarium* in this study it should be regarded as a damaging pathogen of white clover in Waikato pastures.

Previous investigations have demonstrated that different in soil moisture levels and temperatures, both influence the pathogenicity of other root pathogens (McKinney 1923, Graham *et al.* 1957, Crowder and Craigmiles 1960, Cook and Papendick 1972, Barbetti 1984c, Wong *et al.* 1984, 1986), but this is the first of report of these abiotic factors affecting the root rot of a pasture plant by either *C. scoparium, F. crookwellense* or *C. fertilis*.

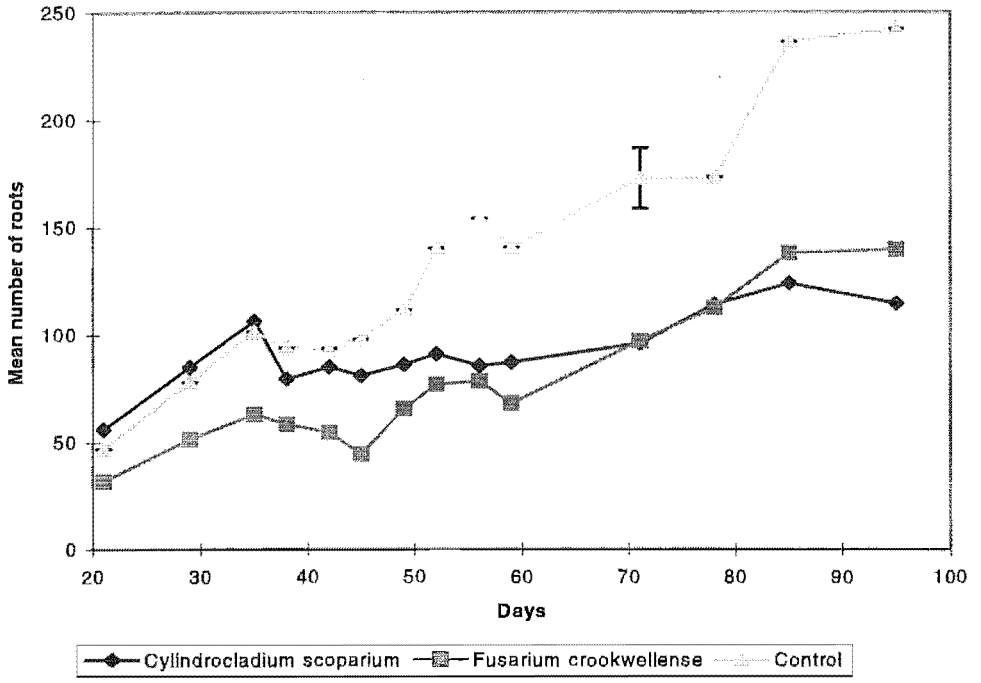
3.3.5 *IN SITU* EXAMINATION OF ROOTS INOCULATED WITH ROOT PATHOGENS USING A MINIRHIZOTRON-BORESCOPE SYSTEM.

Both *C. scoparium* and *F. crookwellense* had a visible impact on the total roots counted along each minirhizotron transect, the cumulative root growth and the yields of both clover and ryegrass. Use of the borescope enabled direct root counts and measurement of root length to be successfully undertaken. The experimental layout is pictured in Figure 3.72.

(a) Root counts.

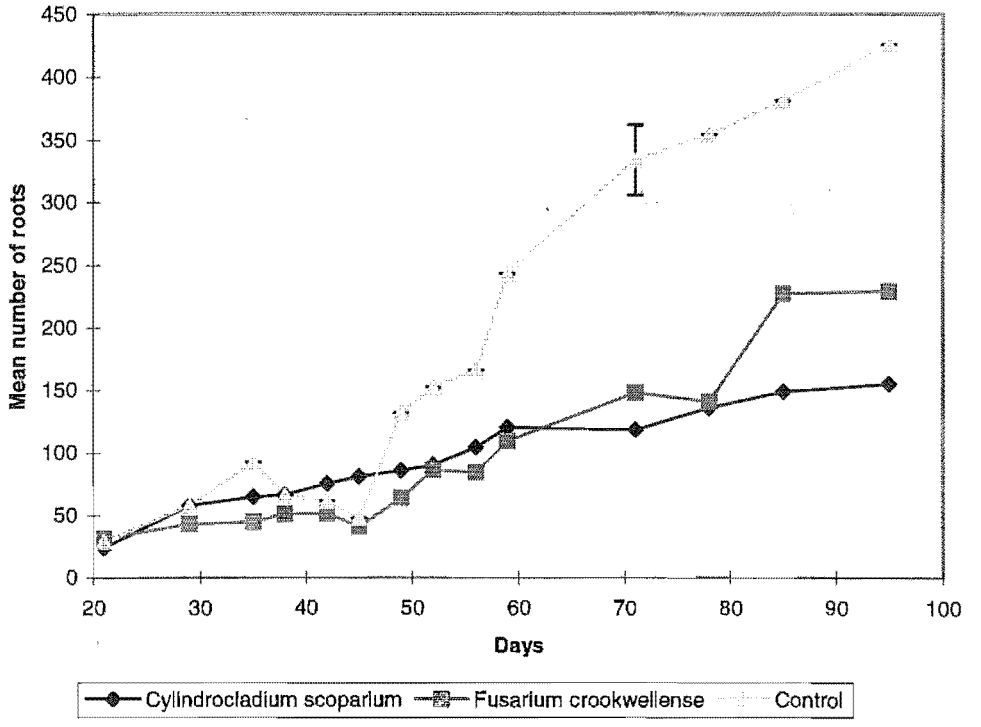
At the completion of this trial the total root count of both clover and ryegrass plants inoculated with the two fungi was significantly lower than the control plants (Figures 3.73, 3.74). Initially there was no difference in root numbers between the three treatments and all mean root count numbers increased in the first three measurements. However, after the inoculation of the two fungal treatments at day 30, the clover root counts of inoculated plants initially decreased compared to the control treatments (Figure 3.73). Mean ryegrass root counts still increased after inoculation but the increase was lower for inoculated plants (Figure 3.74). Overall the total root counts of all plants increased over the 95 days but this increase was larger in control treatments. There was no difference between the root counts of plants inoculated with *C. scoparium* and *F. crookwellense* which indicates they were equally pathogenic to these plants under these conditions.

Figure 3.72 Experimental layout of clover and ryegrass plants grown in the greenhouse and observed using the minirhizotron-borescope system. (A) minirhizotron observation tubes.



Error bar represents SED = 14.1

Figure 3.73 Mean total root count of white clover plants inoculated with *C. scoparium* and *F. crookwellense* using the minirhizotron-borescope observation system.



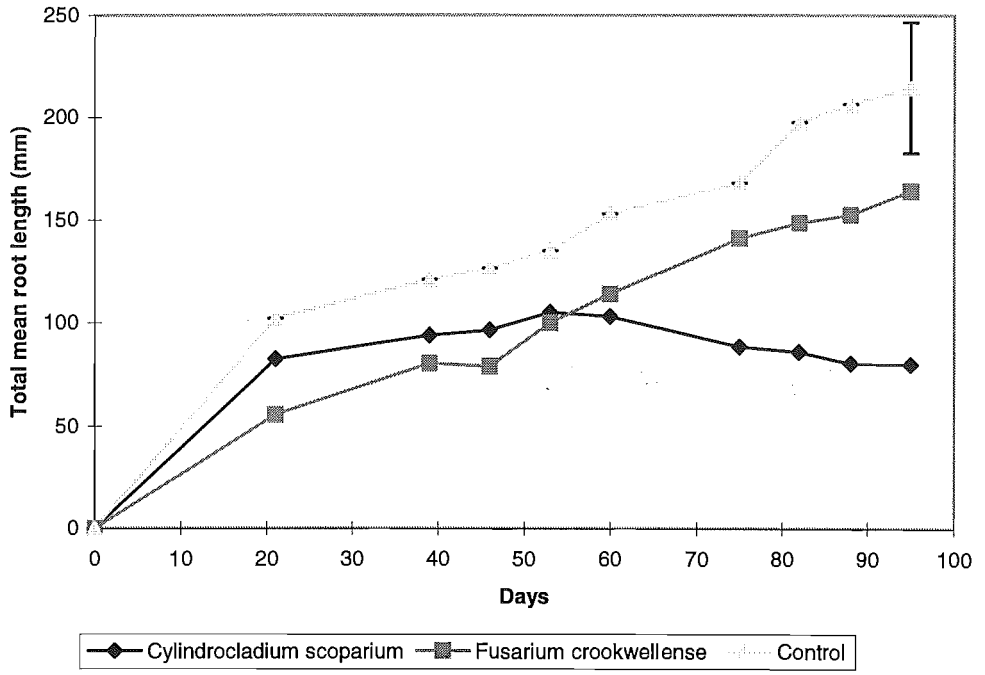
Error bar represents SED = 14.1

Figure 3.74 Mean total root count of perennial ryegrass plants inoculated with *C. scoparium* and *F. crookwellense* using the minirhizotron-borescope observation system.

(b) Clover root growth.

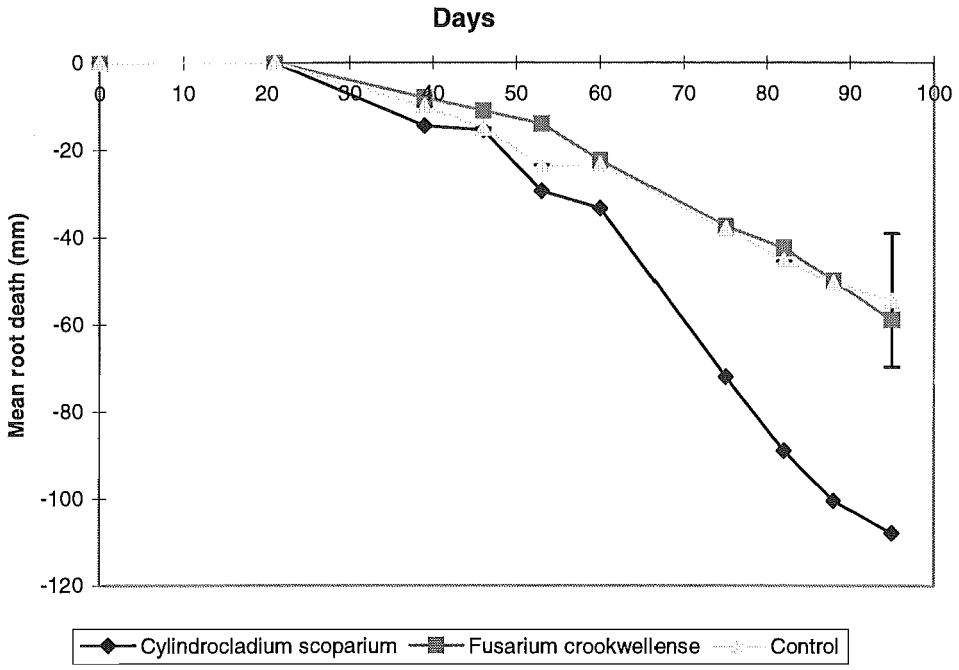
Clover root growth, as measured by the net total root length, was lower in soil inoculated with *C. scoparium* or *F. crookwellense* (Figure 3.75). After 95 days the control root growth had exceeded 200 mm, which was significantly more growth than the 164 mm achieved by roots inoculated with *F. crookwellense* and 80 mm by roots inoculated with *C. scoparium.* The root growth of both control plants and plants inoculated with *F. crookwellense* increased over 95 days which contrasted to the root growth of plants inoculated with *C. scoparium* which decreased over this period.

Mean clover root death was higher in soil inoculated with *C. scoparium* compared to soil containing *F. crookwellense* or control soil (Figure 3.76), which did not differ significantly. Net root growth increased in all treatments (Figure 3.77) but the high root death of plants inoculated with *C. scoparium* led to a lower net root growth than plants inoculated with *F. crookwellense* or control plants.



Error bar represents SED at Day 95 = 32.0.

Figure 3.75 Comparison of the mean total clover root length (mm) of uninoculated control plants to inoculated plants.



Error bar represents SED = 15.3

Figure 3.76 Comparison of the mean total clover root death (mm) of uninoculated control plants to the inoculated plants.

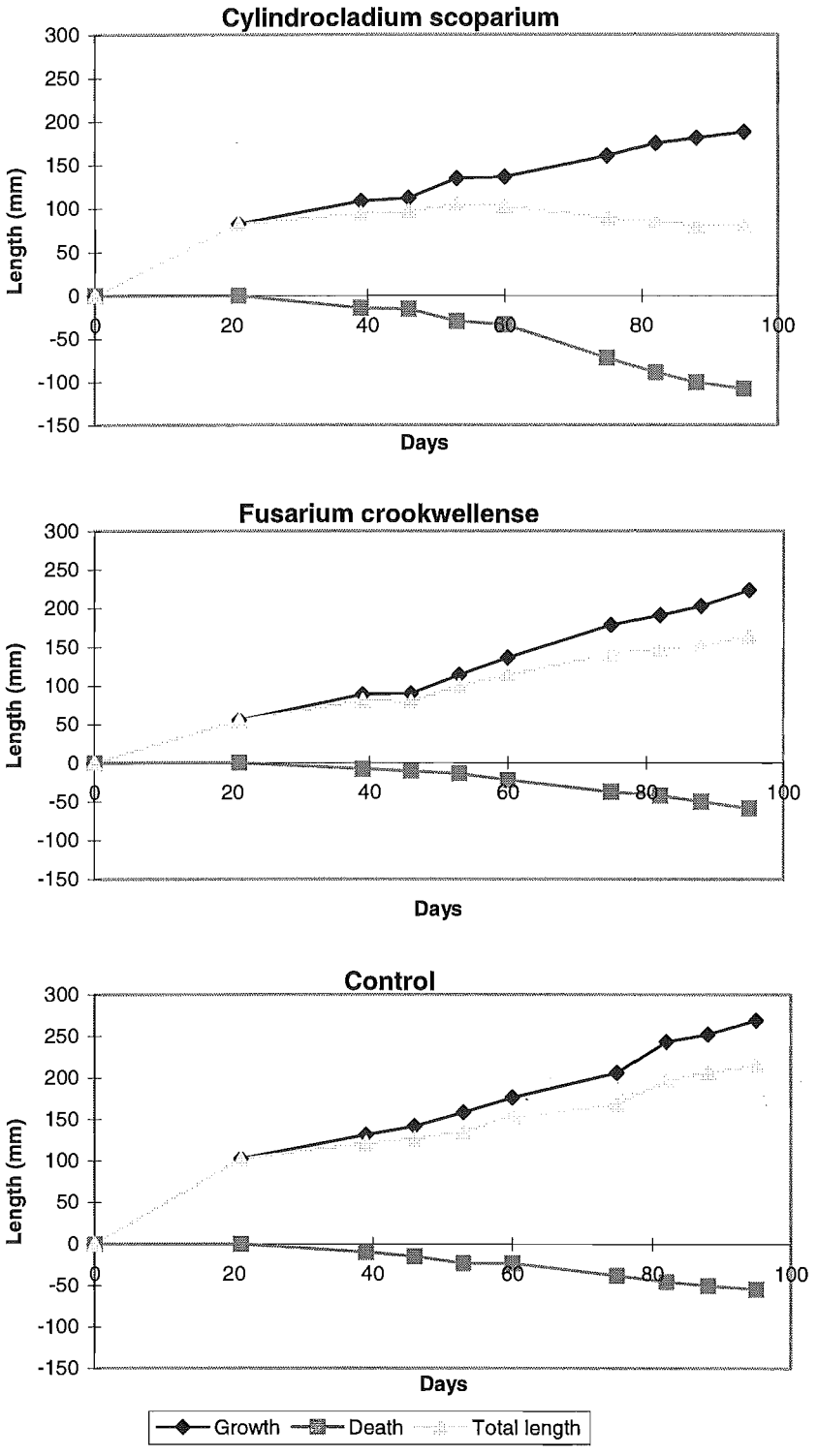
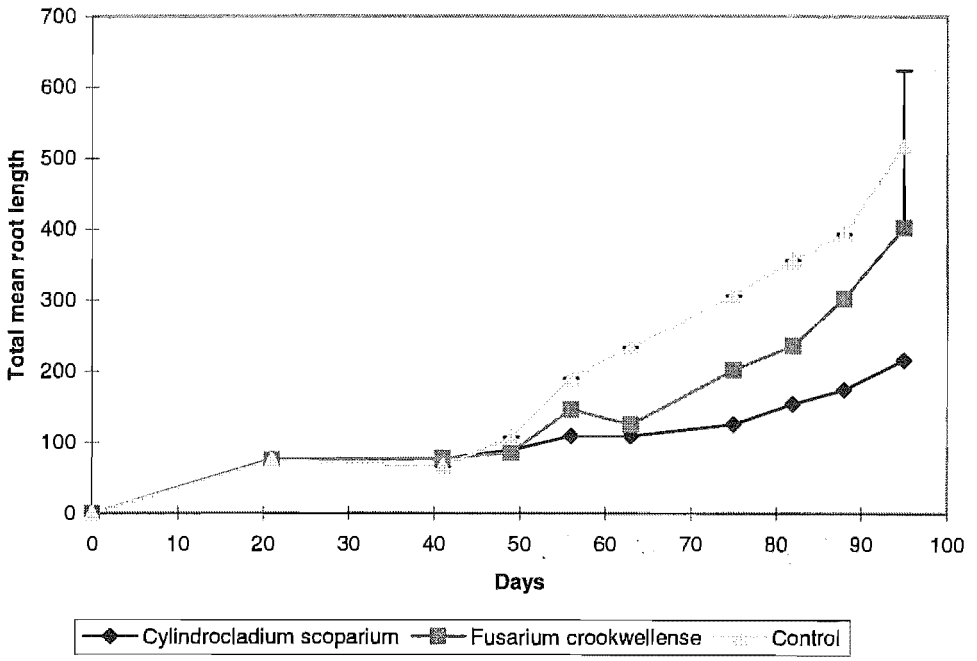


Figure 3.77 Clover root turnover (Growth, Death and total length) of treated and untreated plants.

(c) Ryegrass root growth.

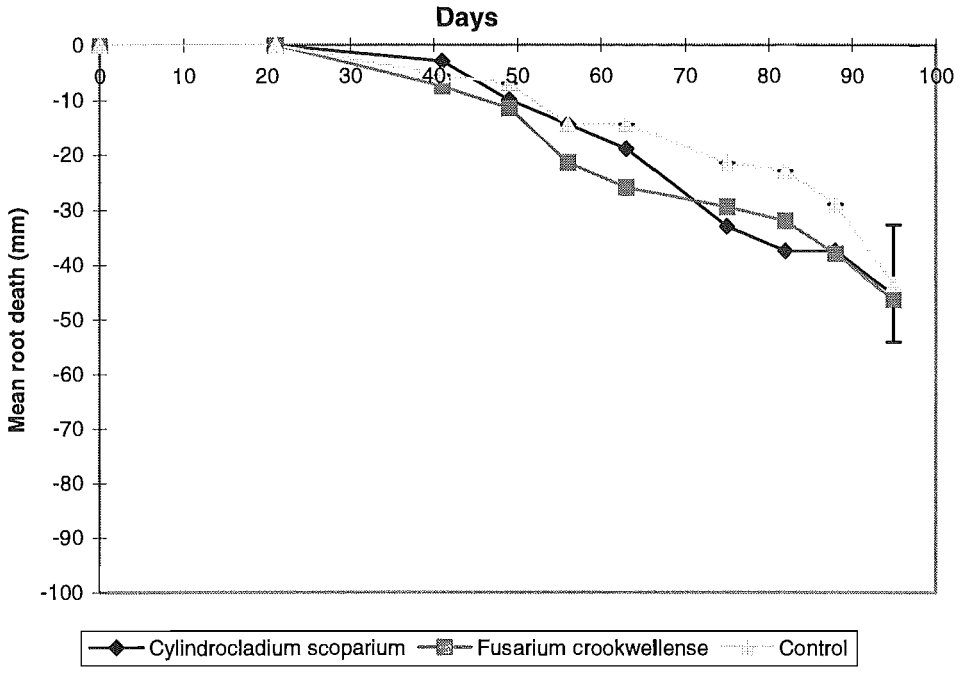
Mean root growth in uninoculated soil reached 516 mm at day 95 which was significantly greater than in soil inoculated with either *F. crookwellense* (402 mm) or *C. scoparium* (216 mm) (Figure 3.78) respectively. Ryegrass root growth was higher for all treatments compared to clover growth. The mean root growth of all three ryegrass treatments increased over the 95 day period, although this increase was much lower in inoculated soils.

There were no differences observed in mean root death among plants grown in inoculated or uninoculated soil (Figure 3.79), however, net growth was lower in inoculated soil than uninoculated soil (Figure 3.80).



Error bar represents SED at Day 95 = 107.1

Figure 3.78 Comparison of the mean total ryegrass root length (mm) of uninoculated control plants to the inoculated plants.



Error bar represents SED at Day 95 = 10.7

Figure 3.79 Comparison of the mean total ryegrass root death (mm) of uninoculated control plants to the inoculated plants.

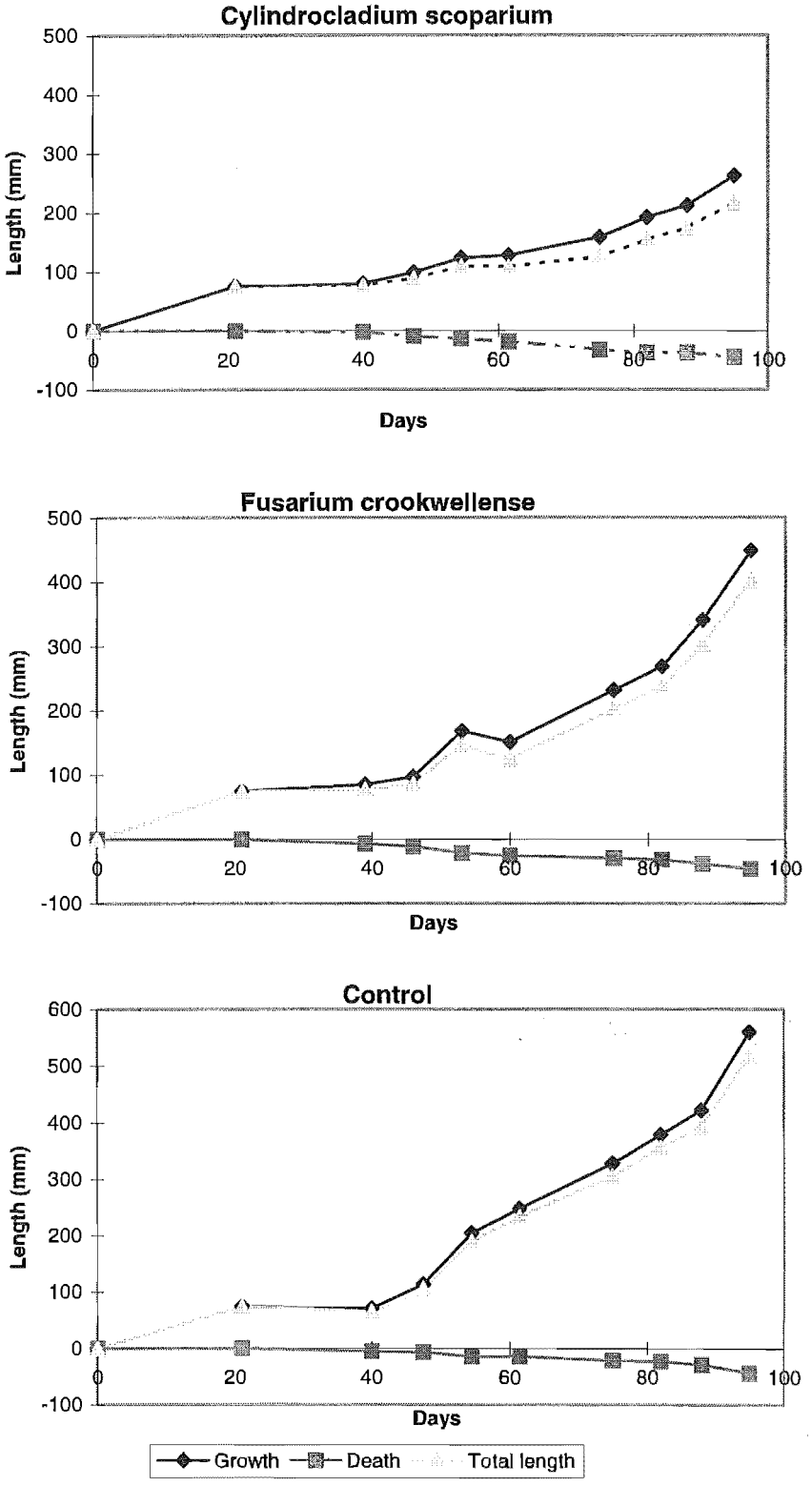
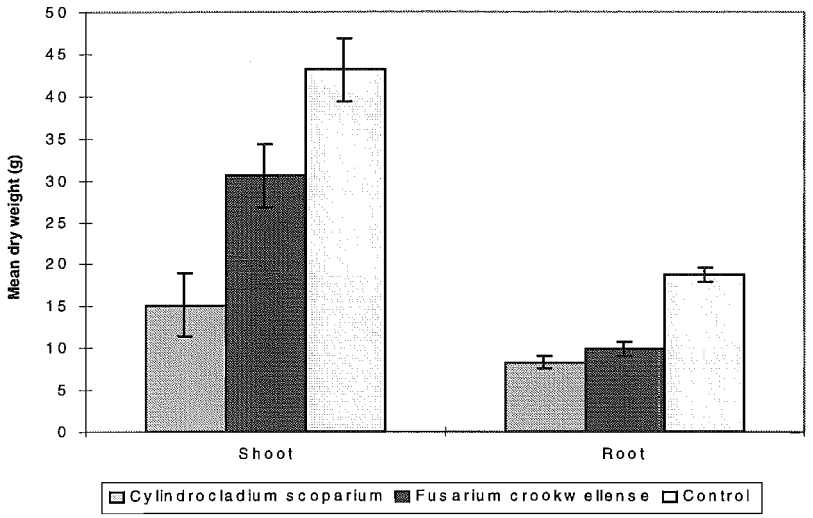


Figure 3.80 Ryegrass root turnover (Growth, Death and total length) of treated and untreated plants.

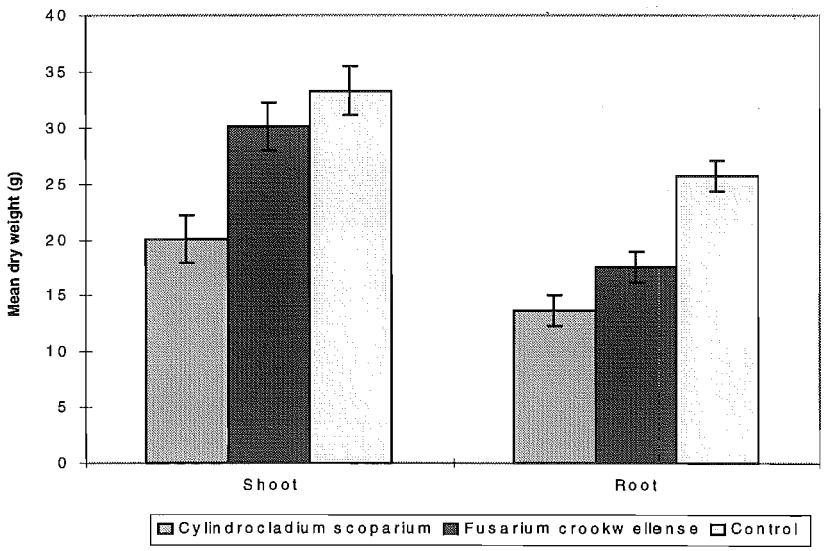
(d) Dry weight yield.

The two pathogens reduced both clover and ryegrass dry weight yields. After 95 days white clover plants in soil inoculated with *C. scoparium* had lower mean dry shoot and root weights compared to control plants (Figure 3.81). Results were similar with *F. crookwellense* except that no significant effect on shoot weight was detected. (Figure 3.81). Similar results were obtained for ryegrass plants (Figure 3.82), as the mean dry root weight was lower in inoculated with *C. scoparium* and *F. crookwellense* than in uninoculated soil. The effect of these two pathogens on both plants are shown in Figures 3.83 - 3.86.



Error bars = SEM, shoot SEM= 7.51, root SEM = 1.56

Figure 3.81 Mean dry weights of white clover plants inoculated with *C. scoparium* and *F. crookwellense* and uninoculated controls.



Error bars = SEM, shoot SEM= 4.17, root SEM = 2.76

Figure 3.82 Mean dry weights of ryegrass plants inoculated with *C. scoparium* and *F. crookwellense* and uninoculated controls.

Figure 3.83 Shoot growth of white clover plants, uninoculated control plants (left) and shoot growth of white clover inoculated with *Cylindrocladium scoparium* (right) at day 95.

Figure 3.84 Shoot growth of white clover plants inoculated with *Fusarium crookwellense* (Left) and shoot growth of an uninoculated control plant (right) at day 95.

Figure 3.85 Shoot growth perennial ryegrass, uninoculated control plants (left), plants inoculated with *Cylindrocladium scoparium* (right) at day 95.

Figure 3.86 Shoot growth of perennial ryegrass, uninoculated control plants (left), plants inoculated with *Fusarium crookwellense* (right) at day 95.

(e) Clover nodulation.

Although a lower number of nodules was observed on inoculated plants than on control plants (Table 3.21), this difference was not significantly different ($P<0.05$), using analysis of variance tests.

Table 3.21 Mean number of nodules on clover roots.

	C. scoparium	*F. crookwellense*	Control	SED
Mean no. of nodules	**449**	**527**	**633**	**187**

Both *C. scoparium* and *F. crookwellense* were reisolated from root segments of both plant species which confirmed them as the causal agents of root disease. They were both absent from control segments which were colonised by *Cladosporium herbarum,* zygomycete fungi, *Trichoderma* spp. and *Fusarium oxysporum.*

Both pathogens decreased clover and ryegrass root numbers and root growth and reduced total plant yields. The effects of *C. scoparium* on clover and ryegrass were substantially greater than *F. crookwellense.* The reduced root growth observed for inoculated plants corresponded to the reduced yields obtained at the end of this experiment. Ryegrass root growth was affected by these two pathogens in that root growth was reduced, but root death did not increase after inoculation, and this allowed root growth to continue to increase but at a slower rate over the measurement period. This contrasted to clover roots inoculated with *C. scoparium* where growth was reduced and root death increased as existing roots were killed faster than they were replaced and this meant clover root counts and growth decreased over the measurement period.

Burgess (1988) reported that *F. crookwellense* was rarely pathogenic to plants, however this study clearly demonstrated that it can be pathogenic to pasture plants by reducing yields and root numbers. *Cylindrocladium scoparium* has previously reported to be pathogenic to pasture plants (Freter and Wilcoxson 1964, Ponnappa *et al.* 1977).

The minirhizotron-borescope system was successfully used to directly measure the effect of fungal pathogens on the root growth of pasture plants. An alternative to the destructive sampling methods this system was useful as it enabled root growth and death to be measured *in situ* over a continuous period without disturbing plant growth and function. The pattern of root growth was also determined, for example, it was possible to observe that *C. scoparium* decreased total clover root growth but that *F. crookwellense* only reduced the rate of increase of clover root growth. The examination of pasture root pathogens in field conditions has to date been difficult and therefore largely ignored, however, future research using this system could be applied to a pasture situation and allow root growth and pathogens to be monitored.

3.4 DISCUSSION

Most of the fungal isolates tested for pathogenicity to pasture species were non-pathogenic root-colonising fungi, however, a minority of fungi present in Waikato pastures were pathogenic to both grass and legume seedlings and plants. Pathogenicity of these fungi was demonstrated by a series of laboratory and pot experiments where seed emergence, plant dry weight yield and root growth were reduced, while disease symptoms and root death were increased compared to the controls and those inoculated with non-pathogenic fungi.

The most important group of pathogens identified were the root-colonising *Fusarium* species. Numerically, this was the most frequently isolated genus from roots, and was found to be highly pathogenic to both legumes and grasses. *Fusarium* spp. were also the most common fungi isolated from germinating pasture seedlings in North Island soils (Falloon 1985a, 1985b). Several of these *Fusarium* species have previously been implicated in pasture plant root death (Leach *et al.* 1963, Leath and Kendall 1978, Holmes 1983, Williams *et al.* 1993) and many species have been isolated from numerous pastures worldwide (Appendix 3).

Another significant pathogen was *Cylindrocladium scoparium.* All pathogenicity tests conclusively found this fungus to be a rapid and aggressive pathogen to seed, seedlings and plants of all tested pasture species. The marked effect of *C. scoparium* on emergence of clovers and lotus compared with grass species indicates that the fungus is more likely to affect establishment of legume plants in pasture than companion grasses. However, the appearance of lesions on seedling roots of grass hosts was indicative of their susceptibility to damage from this pathogen and therefore this fungus could influence growth and survival of these species in Waikato pastures.

The four legume species tested in this study: white clover, red clover, subterranean clover and lotus, were overall more susceptible to attack by pathogenic fungi than most of the grass species. This result was not unexpected as persistence of legumes is a problem in many areas (Kilpatrick and Dunn 1961, Willis 1965, Menzies 1973a, 1973b, Watson *et al.* 1989, Skipp and Christensen 1990, Williams *et al.* 1993) and this poor persistence of pasture legumes has often been attributed to the action of pathogenic root-invading fungi (Leath 1989). Lotus species and annual clover species have been assessed as alternative forage legumes, particularly in dry and low fertility pastures. However the two annual legumes tested here, *Lotus uliginosus* and subterranean clover, appear to be equally susceptible to the pathogens tested as was white and red clover. Reports of establishment and persistence problems of grass species in pastures due to pathogenic root fungi are far less common (Wong 1975, Holmes 1983), although many fungal pathogens of grass

seedlings have been isolated from perennial pastures (Skipp and Hampton 1996, Skipp and Watson 1996). Despite the higher susceptibility of clover seedlings this study clearly demonstrated that common grass species were also susceptible to root damage by many fungi. This may have been largely overlooked previously as damage occurs at a subclinical level in the field.

Temperature and soil moisture are environmental factors which significantly affect the expression of clover root rots. Disease caused by *Codinaea fertilis* was more active at low soil moistures, and its optimum temperature for pathogenic activity was previously reported to be 25°C (Campbell 1982). *Codinaea* root rot has been reported from Gisborne (Menzies 1973a) and the Bay of Plenty (Skipp, Burch pers. comm.) particularly when these areas were susceptible to annual drought stress. *Codinaea* and other pathogens which are active at low soil moistures affect plants that are already stressed and these stresses compound to reduce the tolerance, productivity and survival of these plants in pasture.

The clover root rot activity of *Fusarium crookwellense* was also more severe at low soil moistures and high temperatures. This result was consistent with previous studies where, in general, other species of *Fusarium* were also found to be most active and to survive best in dry soils (Stover 1953). *Fusarium* diseases are frequently more important in soils with a low moisture content (Cook and Papendick 1972). *Fusarium* root rot and damping-off of white clover was also recorded to be most active in dry soils and absent in saturated soil (Graham *et al.* 1957). In contrast, (Wong *et al.* 1984) reported that *F. avenaceum* and *F. oxysporum* caused the most severe subterranean clover root rots at higher soil moistures, but this was in a disease complex combination with other pathogens. Temperature is also a significant factor in *Fusarium* root rot expression in legume crops. Red clover root rot caused by *F. roseum* (now called *F. culmorum*) was more frequent at high temperatures of 28°C or more (Siddiqui and Halisky 1968).

The root rot of *Cylindrocladium scoparium* was most severe at high soil moisture and high temperatures. Overall few root diseases are favoured by low soil moisture, as most root rot and damping off diseases are favoured by wet soils (Cook and Papendick 1972), and this was the case for *C. scoparium*. Nan *et al.* (1991b) also reported that *C. scoparium* was more frequently isolated from red clover roots at high soil moisture (80% WHC) and temperature (25°C). Other common root rots in pasture have been associated with flooding or irrigation where the soil is wet, particularly rots caused by *Pythium, Rhizoctonia, Aphanomyces* and *Thielaviopsis* (Cook and Papendick 1972). Root rot of subterranean clover caused by *Phytophthora clandestina* was most severe at high soil moisture contents (Wong *et al.* 1986). Every microorganism has optimum and minimal soil water potentials for growth, and plant pathogens are no exception. Most root disease occurs in wet soil because low soil moisture is too restrictive for growth of pathogens, and

this is particularly true for pasture diseases where pathogens are generally most active near the soil surface (Cook and Papendick 1972).

Many previous studies of pasture root pathogens record the isolation of these species in combination with other pathogens or pests, so have been described as root rot complexes. This section of work primarily aimed to demonstrate that such fungi are aggressive pasture root pathogens independent of other soil organisms. Although these results were obtained *in vitro,* and results in the field would be moderated by the interaction of other soil and root microbes, the potential for fungi present in Waikato pastures to affect plant productivity and survival has now been shown. Future research in this area should encompass the interactive effects of other deleterious soil organisms and antagonistic or suppressive microorganisms, under both laboratory and field conditions.

Most fungi recorded in the survey appeared to have a broad host range as they were isolated from all plants surveyed. This trend was confirmed by the *in vitro* pathogenicity tests which also clearly showed that most fungi were not host specific and could invade axenically grown seedlings roots of twelve different plant species. This was also reported by (Skipp and Christensen 1989b), who showed fungi commonly found in both white clover and perennial ryegrass reinvaded seedling roots of both hosts. It was also reported that *Bimuria novae zelandiae* and other fungi which were host specific to white clover in the field were not host specific using this *in vitro* seedling test. *Bimuria* was also obtained only from white clover in Waikato pastures and it was observed to reinfect all legume species but was not observed in any grass seedlings. Host specificity in the field may therefore depend on microbial interactions in the rhizosphere such as antagonism, suppression and competition which was absent in these *in vitro* tests which allowed a broader number of hosts to be colonised. As pasture swards are usually a heterogeneous mixture of plants species, the fungi present in pastoral soils would encounter a constantly changing matrix of roots from different species. Therefore host specificity would be a disadvantage for root-colonising fungi in pastures as the chance of encountering a specific host root would be less.

The adverse affect of pathogenic fungi on seed emergence and seedling growth observed in this study has been reported previously in New Zealand (Skipp and Christensen 1981, 1982, 1989a, Falloon 1985) and has implications for current pasture and farm management practices. For example improvement of hill country pasture production includes the introduction of highly productive legumes which increase soil fertility through nitrogen fixation (White 1990), and this is achieved by the oversowing of improved white clover and subterranean clover cultivars over large areas of hill country. Perennial ryegrass and other grasses are also oversown to improve and renew pasture growth. Survival of seed and germinated seedlings is low, but to date this has been mainly attributed to abiotic factors or sward competition (Chapman and Fletcher 1985). This

study shows that there are many pathogenic fungi present in Waikato pastures which have the capacity to reduce and inhibit seedling establishment. This has been confirmed in fungicide trials where seedling germination and survival has increased by up 100% after fungicide application (Michail and Carr 1966, Falloon 1980, Holmes 1983, Nan *et al.* 1992). The pre treatment of seeds with fungicide does not guarantee or afford continuous protection for seeds after being sown (Holmes 1983), thus seedling establishment failure is likely to be in part due to fungal root rot and damping-off.

Although the direction of research here has been focused on the deleterious nature of pathogenic fungi, the importance of non-pathogenic and weakly pathogenic, root-colonising fungi should not be overlooked. Most of the selected fungi initially screened for pathogenicity were non-pathogenic epidermis-colonising fungi. The function of these fungi and their interaction with pathogens and host roots is an important direction for future research. The number of fungi inhabiting roots greatly exceeds that fungi causing major diseases but little is known of their activities (Salt 1979). Infection by this group of fungi did not produce lesions to most inoculated seedlings, even though they invaded root tissues within ten days. Previous evidence suggests that most fungal colonisation of young roots is saprophytic (Dix 1964, Dix and Webster 1995) and that fungal colonisation of older roots is important for root senescence and decomposition (Waid 1957, 1974). Fungal colonisation of old roots is beneficial for plant growth as the invasion of secondary and weak pathogens can increase the decomposition rate of old tissue which in turn increases root turnover and nutrient cycling (Waid 1974). There was some evidence from the pot trials that some species of non-pathogenic fungi, an example being *Mariannaea elegans,* had a beneficial affect on plant growth. Previous studies have shown that some soil fungi antagonise root pathogens, improve plant uptake of minerals and micronutrients, or produce extracellular enzymes such as phosphatase, all of which enhance root growth (Tarafdar and Marschner 1995).

Soilborne pasture root pathogens comprised a significant proportion of the root colonising fungi isolated. Future research must explore the extent to which these fungi lower productivity and survival of pasture plants in the field, after which management practices to improve plant health should be investigated. Potential options espoused for the integrated management of fungal diseases in mixed species pastures include the use of cultivars with greater disease resistance and tolerance to pathogens and other abiotic stress factors, the use of rotations with non-pasture plants adjustment of fertiliser inputs to achieve more vigorous growth, application of biocontrol agents to seed or soil, and modification of grazing management to minimise plant stress (Leath 1989, Skipp and Watson 1996).

Pot trials undertaken in both sterile and non sterile soil, showed that the growth of plants was affected to varying degrees by inoculated pathogenic fungi. A few of the

pathogens studied caused serious plant mortality with conspicuous disease symptoms. In contrast most inoculated fungi, such as *Fusarium solani*, had a sub clinical effect on plants in that there were no obvious disease symptoms but plant growth was affected. The inconspicuous nature of most pasture root diseases is a problem for both pasture pathologists, farmers and the whole agricultural sector, as it has often gone unnoticed. It is therefore harder to achieve a diagnosis than with most crop diseases and it is not perceived as an important area for research. The results presented here clearly demonstrate the adverse affect of these pathogens to both herbage production and root yields.

Root turnover (growth and decay) is a normal feature of plants during their functional life (Wilhelm and Nelson 1970, Deacon 1987, Krauss and Deacon 1994a). It is difficult to establish the primary cause of root death, whether it is endogenously programmed, a response to adverse environmental conditions, or caused by pathogens (Fogel 1983, Deacon 1987, Krauss and Deacon 1994a). Part of the difficulty is that microorganisms invariably colonise senescent roots, so the onset of senescence is accompanied by microbial invasion even if the microbes did not cause it (Deacon and Mitchell 1985, Deacon and Lewis 1986, Deacon 1987, Kirk and Deacon 1987b) Research to date suggests that root turnover is mostly a non-pathogenic seasonal plant process (Wilhelm and Nelson 1970, Krauss and Deacon 1994b). However, the root turnover and decay, of white clover and perennial ryegrass, observed by a minirhizotron-borescope system, increased in the presence of two root pathogens. This led to reduced root growth, increased root death, and decreased herbage productivity, particularly by *C. scoparium.* The preliminary assessment of the minirhizotron method to examine root growth in the presence of soil borne pathogens was achieved and further more extensive trials will be undertaken in the future to determine the seasonal root turnover of pasture plants in the presence of soilborne pathogens under field conditions.

White clover is grown in New Zealand pastures because it has four major benefits; it fixes nitrogen, improves sward quality, complements seasonal growth patterns of its companion grass species, and improves forage intake of animals (Caradus *et al.* 1995). Because of New Zealand's widespread agricultural dependence on this plant, it is of concern that it was shown to be highly susceptible to many commonly occurring soilborne pathogens. Clover health was negatively affected by pathogenic invasion in the variety of tests undertaken. However, more work particularly on the different cultivars grown in the Waikato and host specificity and strain variation of the individual pathogen, need to be investigated to elucidate the problems of white clover persistence. These preliminary results do point to fungal pathogens being a major cause of short term clover survival in perennial pastures.

With the current emphasis on the sustainability of pastures, lower inputs of chemicals, and the use of forages which are less demanding of high fertility, pasture plant

persistence has become an important issue for sustainable production. There needs to be an increased emphasis into research on the factors which influence pasture persistence. Sporadic and conspicuous problems in pasture health have been directly attributed to both individual soilborne fungal root pathogens or a disease complex of several pathogens, but there are also many fungi which remain at a low level within pastures which could be causing an almost invisible but significant effect on pasture production. The range and virulence of root-colonising fungi in Waikato pastures and the potential damage they cause has been demonstrated here, but an even larger amount of work has been identified for further investigation to elucidate the function and influence of root colonising fungi in pastures. In particular pasture root pathology must make the difficult move from the laboratory directly to the field to explore the complete interactions between the mycoflora and roots within the soil.

CONCLUSIONS

A characteristic mycoflora was isolated from Waikato pasture plant roots and although the species identified had diverse systematic affinities, they formed a natural ecological group of root inhabiting fungi. Most fungi isolated were not host specific and could invade the epidermal and cortical tissues of legume and grass species. The largest component of fungi isolated were sterile fungi which were found to be an assemblage of distinct taxonomic groups. These fungi were mostly non pathogenic but actively penetrated pasture roots. Most non-sterile fungi tested for pathogenicity to pasture species were also non-pathogenic, however, a minority of fungi present in Waikato pastures were pathogenic to both grass and legume seedlings and plants. Pathogenicity of these fungi was demonstrated by a series of laboratory and pot experiments where seed emergence, plant dry weight yield and root growth were reduced, while disease symptoms and root death were increased compared to the controls and those inoculated with non-pathogenic fungi. Pathogenic fungi were also found to increase root turnover and were affected by soil moisture and temperature. The pasture legume white clover was particularly susceptible to pathogenic attack by these fungi.

The importance of root-colonising fungi in many of New Zealand's pastoral ecosystems has previously been overlooked. However, the evaluation of fungal biodiversity in Waikato pastures and the potential role of particular fungi to influence the root health of pasture plants have shown that a range fungi can reduce survival and growth of both grasses and legumes. Pastoral mycology is therefore a critical research area for future management practices of agricultural ecosystems.

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APPENDIX 1: Media Recipes.

For all recipes which include agar, Davis bacteriological agar was used. All media were autoclaved at 121°C for 20 minutes. Because of nitrate and other compound contamination in the Hamilton water supply, where most of the media preparation was undertaken, deionised distilled water was always used. The pH of all media were adjusted with either HCl or NaOH to 6.5-7.0, unless otherwise stated.

Bonner and Addicotts medium (BAM)
medium to induce sporulation of *Phymatotrichum omnivorum.*

glucose	20g
KNO_3	0.8g
$MgSO_4.7H_2O$	0.15g
K_2HPO_4	0.8g
KCl	0.087g
$Ca(NO_3)_2 .4 H_2O$	0.01g
agar	15g
distilled water	1000 ml

(the original recipe also added 1mg of ferric tartrate per litre of water)
This medium was adjusted to pH 4.5 with HCl and inoculated plates were incubated for 14 days in the light.

Basal medium 1 (BM1)
Basal medium to assess nitrogen assimilation of yeasts

glucose	1g
K_2HPO_4	1g
$MgSO_4.7H_2O$	0.5g
distilled water	1000 ml

Tube 1: 5ml BM1 broth has no nitrogen added.
Tube 2: Add 0.78g of KNO_3 per 100ml of BM1 broth
Tube 3: Add 0.1g of $(NH_4)_2NO_3$ to 100ml of BM1 broth

Basal medium 2 (BM2)
Basal medium to assess carbon assimilation of yeasts

$(NH_4)_2SO_4$	5g
K_2HPO_4	1g
$MgSO_4.7H_2O$	0.5g
NaCl	0.1g
$CaCl_2.2 H_2O$	0.1g
distilled water	1000 ml
agar	15g

Carbon assimilation medium (CAM) a modification from [Paterson, 1994 #5]

$NH_4H_2 PO_4$	1g
KCl	0.2g
$MgSO_4.7H_2O$	0.2g
$ZnSO_4$	0.02g
distilled water	1000 ml
agar	15g

10 ml of 20% of each Carbon was filter sterilised and added after autoclaving.
pH is adjusted to 5.5 after autoclaving

Czapek Dox Agar (CPZ) IMI method

Czapeks concentrate	50 ml
solution A	50 ml
sucrose	30g
agar	15g
distilled water	1000 ml

Czapek concentrate:

$NaNO_3$	30g
KCl	5g
$MgSO_4.7H_2O$	5g
$FeSO_4.\ 7H_2O$	0.2g
distilled water	1000 ml

Solution A:

K_2HPO_4	20g
distilled water	1000 ml

Czapek Yeast Autolysate Agar (CYA)

K_2HPO_4	0.75g
Czapek concentrate	10 ml
yeast extract	5g
sucrose	30g
agar	15g
distilled water	1000 ml

Glucose Peptone Yeast Agar/Broth (GPY)

glucose	40g
peptone	5g
yeast extract	5g
distilled water	500 ml
agar	15g

Mix ingredients together add agar and dissolve and then autoclave. For GPY broth, omit the agar from the recipe.

Glycerol Nitrate Agar (G25N)

K_2HPO_4	0.75g
Czapek concentrate	7.5 ml
yeast extract	3.7g
glycerol	250g
agar	12g
distilled water	750 ml

Hay Agar (HA)

finely chopped hay pieces	1g
agar	15g
distilled water	1000 ml

Laboratory Potato Dextrose Agar (LPDA)

potato broth	1000 ml
glucose	20g
agar	15g

Potato broth:
Boil 250g of washed and grated potato in 1000ml of water for 15 minutes. Leave to cool, then strain through muslin and autoclave.

Minimum salts medium (MSM)

Na_2HPO_4	6g
KH_2PO_4	3g
NaCl	0.5g
NH_4Cl	1g
distilled water	1000 ml

$MgSO_4.7H_2O$ 1 M	1 ml added after autoclaving
$CaCl_2.2H_2O$ 0.01 M	10 ml added after autoclaving
glucose 20%	10 ml added after autoclaving
thiamine 1%	0.5 ml added after autoclaving

For carbon source assimilation tests the carbon compound is added instead of glucose. For nitrogen source assimilation tests the nitrogen compound (inorganic salts or amino acids) is added instead of NH_4Cl.

Magnesium sulphate solution (0.1M)
Used for long term storage of bacteria.

$MgSO_4.7H_2O$	24.64g
distilled water	1000 ml

Dissolve salt into water and autoclave twice 24 hours apart.

Malt Extract Agar (MEA)

malt extract (maltexo)	20g
agar	15g
distilled water	1000 ml

Dissolve malt extract and agar into the water and autoclave.

Malt Extract Agar Gams method (MEAG)

malt extract (maltexo)	20g
peptone	2.5g
agar	15g
distilled water	1000 ml

Dissolve ingredients and autoclave.

Malt Extract Agar Pitts method (MEAP)

malt extract	20g
peptone	1g
glucose	20g

agar	15g
distilled water	1000 ml

Dissolve ingredients and autoclave.

Nitrogen assimilation medium (NAM)

KH_2PO_4	1g
KCl	0.2g
$MgSO_4.7H_2O$	0.2g
$CaCl_2.2H_2O$	0.1g
Glucose	10g
distilled water	1000 ml
agar	15g

1g of each Nitrogen source was added

Oat Agar (OA)

oatmeal	40g
agar	15g
distilled water	1000 ml

Soak the oatmeal in the water for 24 hr and then drain through muslin, dissolve agar and autoclave.

Potato Carrot Agar (PCA) IMI method.

washed, peeled, grated potato	20g
washed, peeled, grated carrot	20g
Davis bacteriological agar	15g
distilled water	1000 ml

Boil vegetables for 1 hour in tap water, strain through a fine sieve and push through muslin. Add agar to broth and dissolve, then autoclave at 121°C for 20 minutes.

Potato Dextrose Agar (PDA)

Difco potato dextrose agar	39g
distilled water	1000 ml

Sabouraud Agar (SAB)

glucose	50g
peptone	10g
agar	15g
distilled water	1000 ml

Soil Extract Agar (SEA, Nemec 1969)

soil	500g
K_2HPO_4	0.2g
agar	15g
distilled water	1000 ml

Add 500g of soil to 1200ml of water and autoclave at 121°C for 30 minutes. Leave overnight then centrifuge for 10 minutes. Pour off supernatant to make up 1000ml and add K_2HPO_4 and agar. Heat in microwave for 5 minutes and adjust pH to 7.2. Autoclave at 121°C for 20 minutes.

Sterile Saline Solution (SSS)

Na Cl	8.5g
distilled water	100 ml

Sucrose Asparagine Agar (SUC)

sucrose	100g
asparagine	10g
KCl	0.125g
$MgSO_4.7H_2O$	0.25g
K_2HPO_4	0.25g
$Ca(NO_3)$	1g
$FeSO_4$	0.033g
$ZnSO_4$	0.027g
Cysteine	0.01g
yeast extract	0.1g
agar	15g
distilled water	1000 ml

Tryptic Soy Agar (TSA)

Gibco BRL tryptic soy agar	40g
distilled water	1000 ml

V8 Agar (V8)

V8 vegetable juice	200 ml
agar	20g
distilled water	800 ml

V8 broth medium (VBM)

V8 vegetable juice	100 ml
D-glucose	10g
Bacto-peptone	2g
chloramphenicol	0.05g
$Fe\ Cl_2$	0.01g
KCl	0.25g
$MgSO_4.7H_2O$	0.5g
K_2HPO_4	1g
distilled water	900 ml

Water Agar (WA)

Davis bacteriological agar	15g
distilled water	1000 ml

Yeast Extract Agar (YEA)

yeast extract	3g
glucose	10g
$MgSO_4.7H_2O$	0.05g
K_2HPO_4	0.1g
agar	15g
coconut milk	50 ml
distilled water	950 ml

APPENDIX 2: Complete list of species isolated from sampled pasture plants.

I Survey One. Total number and percentage of fungal species isolated from surface sterilized root segments of sweet vernal, browntop, white clover and perennial ryegrass from Whatawhata hill country pastures.

PLANT:	Sweet vernal	Browntop	Clover	Rvegrass	Total	Total
FUNGAL SPECIES:					count	%
Acremoniella atra	-	-	2	-	**2**	0.05
Acremonium curvulum	5	8	10	7	**30**	0.75
Acremonium fusidioides	2	7	2	3	**14**	0.35
Acremonium kiliense	6	4	6	4	**20**	0.50
Acremonium murorum	1	4	-	-	**5**	0.12
Acremonium sp.	-	-	4	2	**6**	0.15
Acremonium strictum	1	-	1	1	**3**	0.07
Alternaria alternata	-	2	2	-	**4**	0.10
Aphanocladium album	5	-	-	3	**8**	0.20
Arthrinium arundinis	3	1	5	-	**9**	0.22
Arthrobotrys oligospora	-	1	1	3	**5**	0.12
Aspergillus fumigatus	-	1	-	-	**1**	0.02
Aspergillus niger	3	-	1	-	**4**	0.10
Aureobasidium pullulans	3	-	6	3	**12**	0.30
Beauveria bassiana	-	-	1	-	**1**	0.02
Bimuria novae-zelandiae	-	-	19	-	**19**	0.47
Bipolaris sp.	-	-	1	-	**1**	0.02
Botrytis cinerea	-	2	1	4	**7**	0.17
Chaetomium funicola	6	8	3	6	**23**	0.57
Chaetomium globosum	-	2	-	3	**5**	0.12
Chaetomium indicum	-	-	-	1	**1**	0.02
Chrysosporium	-	-	1	-	**1**	0.02
Cladosporium herbarum	5	3	6	7	**21**	0.52
Clasterosporium sp.	1	-	-	2	**3**	0.07
Codinaea fertilis	56	62	134	78	**330**	**8.20**
Colletotrichum sp.	4	1	11	-	**16**	0.40
Curvularia trifolii	-	1	-	2	**3**	0.07
Cylindrocarpon destructans	1	6	26	15	**48**	**1.19**
Cylindrocladium scoparium	-	-	31	-	**31**	0.77
Dactylaria acerosa	20	13	18	16	**67**	**1.67**
Dreschlera dematioidea	6	3	2	-	**11**	0.27
Epicoccum nigrum	1	1	2	3	**7**	0.17
Fusarium acuminatum	1	2	2	4	**9**	0.22
Fusarium avanaceum	9	2	27	1	**39**	0.97
Fusarium crookwellense	6	7	14	12	**39**	0.97
Fusarium culmorum	1	1	3	4	**9**	0.22
Fusarium equiseti	-	-	-	1	**1**	0.02
Fusarium graminum	2	-	-	-	**2**	0.05
Fusarium oxysporum	42	98	147	102	**389**	**9.67**
Fusarium sambucinum	1	-	-	-	**1**	0.02
Fusarium solani	1	4	2	5	**12**	0.30
Fusarium tricinctum	2	-	-	-	**2**	0.05
Geotrichum candidum	1	-	-	-	**1**	0.02
Gliocladium roseum	17	15	29	38	**99**	**2.46**

Fungal species	Sweet vernal	Browntop	Clover	Ryegrass	Total	Total
Gliocladium sp 1	1	-	-		**1**	0.02
Gliocladium sp 2	-	-	-	1	**1**	0.02
Gongronella butleri	2	10	1	1	**14**	0.35
Idriella bolleyi	9	1	5	1	**16**	0.40
Idriella lunata	-	-	-	1	**1**	0.02
Mariannaea elegans	3	1	7	8	**19**	0.47
Metarhizium anisopliae				-	**2**	0.05
Mortierella alpina	-	-	-	3	**3**	0.07
Mortierella elongata	2	4	-	-	**6**	0.15
Mortierella gamsii	-	5	2	2	**9**	0.22
Mortierella globulifera	5	3	9	13	**30**	0.75
Mortierella hyalina	1	-	-	-	**1**	0.02
Mucor sp.	3	3	3	4	**13**	0.32
Myrothecium verrucaria	-	-	5	5	**10**	0.25
Paecilomyces carneus	5	9	10	4	**28**	0.70
Paecilomyces lilacinus	9	12	6	12	**39**	0.97
Paecilomyces marquandii	-	-	1	2	**3**	0.07
Penicillium atramentosum	-	-	4	-	**4**	0.10
Penicillium brevicompactum	2	1	1	4	**8**	0.20
Penicillium chrysogenum	1	13	3	2	**19**	0.47
Penicillium citrinum	-	2	-	-	**2**	0.05
Penicillium crustosum	-	-	-		**2**	0.02
Penicillium decumbens	-	1	-	-	**1**	0.02
Penicillium islandicum	1	-	-		**1**	0.02
Penicillium janczewskii	-	-	-	1	**1**	0.02
Penicillium janthinellum	4	35	4	26	**69**	**1.72**
Penicillium minioluteum	-	1	-	-	**1**	0.02
Penicillium simplicissimum	13	15	6	15	**49**	1.22
Penicillium sp.	-	1	5	-	**6**	0.15
Penicillium variabile	-	1	-	-	**1**	0.02
Periconia macrospinosa	29	7	9	6	**51**	**1.27**
Pithomyces chartarum	1	1	-	1	**3**	0.07
Plectosporium tabacinum	3	1	5	4	**13**	0.32
Preussia aemulans	6	-	-	-	**6**	0.15
Pseudallescheria boydii	1	-	-	-	**1**	0.02
Pycnidial fungi	23	11	14	12	**60**	**1.49**
Pyricularia oryzae	4	1	1	-	**6**	0.15
Ramichloridium schultzeri	-	-	8	-	**8**	0.20
Rhizoctonia solani	4	7	5	6	**22**	0.35
Sporothrix schenckii	-	1	-	-	**1**	0.02
Sterile dark fungi	144	94	47	95	**294**	**7.31**
Sterile dark group 1	12	2	6	2	**22**	0.55
Sterile dark group 2	1	-	-	1	**2**	0
Sterile dark group 3	21	10	18	9	**144**	**1.23**
Sterile dark group 4	8	121	1	22	**152**	**3.78**
Sterile dark group 5	83	69	106	96	**354**	**8.80**
Sterile dark group 6	86	75	13	96	**270**	**6.71**

Fungal species	Sweet vernal	Browntop	Clover	Ryegrass	Total	Total
Sterile dark group 7	30	64	7	58	**159**	**3.95**
Sterile dark group 8	23	3	13	7	**47**	0.68
Sterile hyaline fungi	23	43	14	40	**120**	**2.98**
Sterile hyaline group 1	25	10	6	7	**48**	**1.19**
Sterile hyaline group 2	-	-	-	4	**4**	0.10
Sterile hyaline group 3	84	125	11	33	**282**	**4.09**
***Tetracladium* sp.**	-	-	1	-	**1**	0.02
Thozetella tocklaiensis	15	11	-	3	**29**	0.72
***Tolypocladium* sp.**	-	1	-	-	**1**	0.02
***Trichobotrys* sp.**	-	1	-	-	**1**	0.02
Trichoderma hamatum	9	30	23	39	**101**	**2.51**
Trichoderma harzianum	5	2	5	4	**16**	0.40
Trichoderma koningii	2	5	2	10	**19**	0.47
Trichoderma polysporum	5	-	5	10	**20**	0.50
Trichoderma psuedokoningii	1	1	-	1	**3**	0.07
***Trichoderma* sp.**	-	4	-	2	**7**	0.17
Trichosporon cutaneum	10	2	15	10	**37**	0.92
Unidentified species	2	3	7	3	**15**	0.37
***Verticicladiella* sp.**	4	1	-	1	**6**	0.15
Verticillium albo-atrum	-	-	1	-	**1**	0.02
Verticillium cephalosporum	-	1	-	-	**1**	0.02
Verticillium chlamydosporium	8	1	7	3	**11**	0.27
Verticillium dahliae	-	10	5	12	**27**	0.67
TOTAL	**946**	**1096**	**948**	**1033**	**4023**	**100**
Total no. of species isolated	**61**	**65**	**63**	**61**	**100**	

II Survey Two. Total number and percentage of fungal species isolated from surface sterilized root segments of perennial ryegrass and white clover from Ruakura dairy pastures.

Pasture plant	Ryegrass	Clover	Total	Total
FUNGAL SPECIES:			**count**	**%**
Acremonium curvulum	12	7	**19**	0.66
Acremonium fusidioides	1	1	**2**	0.07
Acremonium kiliense	4	5	**9**	0.31
***Acremonium* sp.**	5	3	**8**	0.28
Acremonium strictum	2	5	**7**	0.24
Alternaria alternata	3	0	**3**	0.10
Amorphotheca resinae	1	0	**1**	0.03
Arthrobotrys oligospora	1	1	**2**	0.07
Aspergillus fumigatus	0	3	**3**	0.10
Aspergillus glaucus	1	0	**1**	0.03
Aspergillus niger	16	0	**16**	0.56
Aspergillus ustus	1	0	**1**	0.03
Aureobasidium pullulans	2	13	**15**	0.52
Bimuria novae-zelandiae	0	62	**62**	**2.16**
***Botryosporium* sp.**	0	1	**1**	0.03
Botrytis cinerea	1	0	**1**	0.03
Chaetomium funicola	0	3	**3**	0.10

Fungal species	Ryegrass	Clover	Total	Total
Chaetomium globosum	1	1	**2**	0.07
Chrysosporium	1	5	**6**	0.21
Cladosporium herbarum	9	4	**13**	0.45
Clasterosporium sp.	5	0	**5**	0.17
Codinaea fertilis	99	229	**328**	**11.40**
Colletotrichum sp.	7	3	**10**	0.35
Cylindrocarpon destructans	49	42	**91**	**3.16**
Cylindrocladium scoparium	6	54	**60**	**2.09**
Dactylaria acerosa	0	4	**4**	0.14
Dreschlera dematioidea	4	0	**4**	0.14
Epicoccum nigrum	5	3	**8**	0.28
Fusarium acuminatum	2	3	**5**	0.17
Fusarium avanaceum	25	31	**56**	**1.95**
Fusarium crookwellense	28	35	**63**	**2.19**
Fusarium culmorum	47	10	**57**	**1.98**
Fusarium oxysporum	136	247	**383**	**13.32**
Fusarium solani	12	17	**29**	**1.01**
Gliocladium roseum	24	31	**55**	**1.91**
Gliocladium sp 1	2	2	**4**	0.14
Gongronella butleri	20	13	**33**	**1.15**
Humicola fuscoatra	0	1	**1**	0.03
Idriella bolleyi	18	7	**25**	0.87
Mariannaea elegans	7	3	**10**	0.35
Mortierella alpina	3	2	**5**	0.17
Mortierella bainieri	1	1	**2**	0.07
Mortierella elongata	4	4	**8**	0.28
Mortierella gamsii	14	5	**19**	0.66
Mortierella globulifera	5	7	**12**	0.42
Mortierella hyalina	1	0	**1**	0.03
Mucor sp.	14	13	**27**	0.94
Myrothecium verrucaria	3	1	**4**	0.14
Paecilomyces carneus	5	4	**9**	0.31
Paecilomyces lilacinus	15	4	**19**	0.66
Paecilomyces marquandii	8	0	**8**	0.28
Penicillium atramentosum	2	0	**2**	0.07
Penicillium brevicompactum	0	1	**1**	0.03
Penicillium chrysogenum	8	3	**11**	0.38
Penicillium citrinum	1	0	**1**	0.03
Penicillium crustosum	1	1	**2**	0.07
Penicillium decumbens	2	0	**2**	0.07
Penicillium griseofulvum	1	0	**1**	0.03
Penicillium islandicum	1	0	**1**	0.03
Penicillium janthinellum	6	1	**7**	0.24
Penicillium simplicissimum	4	3	**7**	0.24
Periconia macrospinosa	17	10	**27**	0.94
Pestalotia sp.	1	0	**1**	0.03
Phymatotrichum omnivorum	1	0	**1**	0.03
Plectosporium tabacinum	2	2	**4**	0.14
Preussia aemulans	18	0	**18**	0.63

Fungal species	Ryegrass	Clover	Total	Total
Pseudallescheria boydii	11	0	**11**	0.38
Pycnidial fungi	33	35	**68**	**2.36**
Pyrenophora **sp.**	3	0	**3**	0.10
Ramichloridium schultzeri	15	9	**24**	0.83
Rhizoctonia solani	1	1	**2**	0.07
Sordaria fimicola	1	0	**1**	0.03
Sporothrix schenckii	1	0	**1**	0.03
Sterile dark fungi	111	58	**169**	**5.88**
Sterile dark group 1	15	19	**34**	1.18
Sterile dark group 2	6	2	**8**	0.28
Sterile dark group 3	18	9	**27**	0.94
Sterile dark group 4	15	5	**20**	0.70
Sterile dark group 5	213	102	**315**	**10.95**
Sterile dark group 6	24	24	**48**	**1.67**
Sterile dark group 7	109	13	**122**	**4.24**
Sterile dark group 8	0	1	**1**	0.03
Sterile hyaline fungi	60	17	**77**	**2.68**
Sterile hyaline group 1	49	10	**59**	**2.05**
Sterile hyaline group 2	0	1	**1**	0.03
Sterile hyaline group 3	5	24	**29**	**1.01**
Tetraploa aristata	0	1	**1**	0.03
Thielaviopsis basicola	0	6	**6**	0.21
Trichoderma hamatum	13	20	**33**	**1.15**
Trichoderma harzianum	14	15	**29**	**1.01**
Trichoderma koningii	19	20	**39**	**1.36**
Trichoderma polysporum	55	9	**64**	**2.23**
Trichoderma psuedokoningii	6	1	**7**	0.24
Trichoderma **sp.**	3	0	**3**	0.10
Trichoderma viride	2	0	**2**	0.07
Trichosporon cutaneum	5	5	**10**	0.35
Unidentified species	5	1	**6**	0.21
Verticicladiella **sp.**	5	2	**7**	0.24
Verticillium chlamydosporium	6	19	**25**	0.87
Verticillium dahliae	4	10	**14**	0.49
TOTAL	**1526**	**1350**	**2876**	**100**
Total no. of species isolated	**76**	**62**	**86**	

III Survey Three. Total number and percentage of fungal species isolated from surface sterilized root segments of Yorkshire fog, lotus, subterranean clover and soft brome.

Pasture plant	Yorkshire fog	Lotus	Subterranean clover	Soft Brome		
Fungal species:					**Total**	**Total%**
Alternaria alternata	-	1	-	-	**1**	0.3
Arthrinium arundinis	-	-	-	1	**1**	0.3
Arthrobotrys oligospora	-	-	1	-	**1**	0.3
Aspergillus niger	4	1	1	-	**6**	**1.8**
Aspergillus ustus	-	-	-	1	**1**	0.3
Botrytis cinerea	1	-	-	-	**1**	0.3
Chaetomium globosum	-	-	-	2	**2**	0.6
Cladosporium herbarum	-	1	-	-	**1**	0.3
Codinaea fertilis	8	20	23	-	**51**	**15.32**
Colletotrichum **sp.**	-	1	-	-	**1**	0.3
Cylindrocladium scoparium	-	-	1	-	**1**	0.3
Dactylaria acerosa	1	1	-	2	**4**	**1.2**
Epicoccum nigrum	1	-	-	-	**1**	0.3
Fusarium avenaceum	1	-	5	1	**7**	**2.1**
Fusarium crookwellense	-	1	1	-	**2**	0.6
Fusarium culmorum	2	5	-	-	**7**	**2.1**
Fusarium equiseti	-	-	-	1	**1**	0.3
Fusarium oxysporum	14	19	7	6	**46**	**13.82**
Fusarium tricinctum	1	-	2	-	**3**	0.9
Gliocladium roseum	-	3	1	1	**5**	**1.5**
Gongronella butleri	-	1	3	-	**4**	**1.2**
Idriella bolleyi	1	1	7	-	**9**	**2.7**
Mariannaea elegans	-	-	3	4	**7**	**2.1**
Mortierella alpina	-	-	-	3	**3**	0.9
Mortierella gamsii	-	-	-	2	**2**	0.6
Mortierella elongata	-	-	1	-	**1**	0.3
Mucor **sp.**	2	-	1	-	**3**	0.9
Paecilomyces carneus	2	-	-	1	**3**	0.9
Paecilomyces lilacinus	2	2	-	-	**4**	**1.2**
Penicillium brevicompactum	-	1	-	-	**1**	0.3
Penicillium chrysogenum	-	-	-	5	**5**	**1.5**
Penicillium dendriticum	-	-	-	1	**1**	0.3
Penicillium expansum	-	-	1	-	**1**	0.3
Penicillium janthinellum	2	-	2	2	**6**	**1.8**
Penicillium oxalicum	-	1	-	-	**1**	0.3
Penicillium rastrickii	-	-	1	-	**1**	0.3
Penicillium rugulosum	-	-	1	-	**1**	0.3
Penicillium simplicissimum	-	-	1	3	**4**	**1.2**
Periconia macrospinosa	18	14	9	5	**46**	**13.82**
Pycnidial fungi	5	1	-	1	**7**	**2.1**
Sterile dark fungi	10	6	3	5	**24**	**7.2**
Sterile hyaline fungi	5	-	7	6	**18**	**5.41**
Trichoderma hamatum	-	2	2	7	**11**	**3.3**
Trichoderma harzianum	4	-	2	3	**9**	**2.4**
Trichoderma koningii	1	-	3	-	**4**	**1.2**
Trichoderma polysporum	3	-	-	3	**6**	**1.8**
Tricellula **sp.**	-	-	6	-	**6**	**1.8**
Verticillium lecanii	-	-	3	-	**3**	0.9
TOTAL	**88**	**82**	**97**	**66**	**333**	**100**
Total no. species isolated	**17**	**17**	**25**	**20**	**45**	

APPENDIX 3:

Isolation index and checklist of previous host records of fungal species isolated from Waikato pastures.

Three surveys of 40 Waikato pasture plots were undertaken to isolate root-colonising fungi from the dominant pasture species of low fertility hill country pasture and high fertility dairy pasture. Survey one sampled four dominant pasture plants (white clover *Trifolium repens* L., perennial ryegrass, *Lolium perenne* L., sweet vernal *Anthoxanthum odoratum* L., and browntop *Agrostis capillaris* L.), from 20 plots at Whatawhata hill country pasture sites. Survey two sampled two dominant pasture plants (white clover and perennial ryegrass) from 20 plots at Ruakura improved dairy pastures sites. Survey three sampled four additional companion sub-dominant pasture plants (Yorkshire fog *Holcus lanatus* L., lotus *Lotus uglinosus* L., subterranean clover *Trifolium subterraneum* L. and soft brome *Bromus hordeaceus* L.), from the 20 hill country plots at Whatawhata. Over 7232 isolates were obtained from these surveys, and a total of 118 species of fungi were identified from surface sterilised root segments. An index was compiled to summarise the isolation frequency, site and host plant, for each fungal species. Information on previous isolation and host records for all fungi is also included, with particular reference to reported New Zealand and pasture records. An *in vitro* Petri plate test was carried out to assess the pathogenicity and root colonisation of 70 fungal species isolated in these surveys (see Section two). Each isolate was inoculated onto the seedling roots of 12 common pasture plant species (Table 1), grown axenically on water agar (WA). The results of this *in vitro* screen are also reported in this index along other pathogenicity reports published in the literature.

Table 1. Twelve plant species screened *in vitro* for susceptibility to 70 fungal species

Common name	Species name	Grasslands cultivar/code
White clover	*Trifolium repens* L.	Huia
Lotus	*Lotus uliginosus* Schkuhr	Maku
Subterranean clover	*Trifolium subterraneum* L.	AK1003
Red clover	*Trifolium pratense* L.	Pawera
Timothy	*Phleum pratense* L.	Kahu
Yorkshire fog	*Holcus lanatus* L.	Massey Basyn
Browntop	*Agrostis capillaris* L.	Egmont
Sweet vernal	*Anthoxanthum odoratum* L.	BZ 2330
Tall fescue	*Festuca arundinacea* L.	Roa
Cocksfoot	*Dactylis glomerata* L.	Wana
Perennial ryegrass	*Lolium perenne* L.	Nui endophyte-free
Soft brome (Goosegrass)	*Bromus hordeaceus* L. (= *B. mollis*)	Whatawhata wild type

1. *Acremoniella atra* Sacc.

Isolation site: Whatawhata

Host: *Trifolium repens*

This hyphomycete fungus was rare, being isolated twice from white clover at Whatawhata. There are no previous isolation records from pasture roots in New Zealand, however it has been isolated from leaves of ryegrass in pastures (Thomas and Shattock 1986). This fungus was found to be non-pathogenic to the seedlings of pasture grasses and clovers but was able to colonise cortical and epidermal cells of seedlings, without producing disease symptoms. It has been previously reported from roots and stems of cereals (Khatskevich and Benken 1990) and maize (Kruger 1976), where pathogenicity tests also showed it to be saprophytic. *Acremoniella atra*, has been found to be antagonistic to root and storage rot pathogens (Hoeven *et al.* 1979; Rod 1984), and has been investigated as a potential mycoparasite for biocontrol against diseases such as black root rot of cucumber (Hoeven *et al.* 1979).

***Acremonium* Link ex Fr.**

This hyphomycete genus was commonly isolated from pasture roots of sweet vernal, white clover, ryegrass and browntop at both Ruakura and Whatawhata pasture sites. A total of 123 isolates (1.78%) of this genus were obtained in survey one and two. Five species were identified (see entries 2-6 below) with a further 14 isolates unidentified to species. Most research into the significance of *Acremonium* in pastures has been in relation to its occurrence as a 'strict' endophyte of important grass species, and its consequent effect on livestock and pasture production. However, this genus has also previously been isolated in New Zealand pastures from the roots of red clover (Skipp 1986), white clover (Sarathchandra *et al.* 1995, Skipp and Christensen 1982, Skipp and Christensen 1983, Skipp *et al.* 1982), perennial ryegrass (Falloon 1985, Skipp and Christensen 1989, Sarathchandra *et al.* 1995). Some species of *Acremonium* can cause root rots while others are vascular wilt pathogens (Kommedahl and Windels 1979).

2. *Acremonium curvulum* (Corda) W. Gams (=*Gliomastix murorum* (Corda) Hughes.

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris.*

Acremonium curvulum was the most common hyaline *Acremonium* species isolated, with 49 isolates being obtained. It was present at all sites, being obtained from the roots of perennial ryegrass, white clover, sweet vernal and browntop. In pathogenicity tests it was found to be a weak pathogen of subterranean clover, red clover, white clover, sweet vernal and browntop and could invade epidermal and cortical cells of all pasture seedlings, except soft brome. Active and intercellular invasion by hyphae was observed on infected roots.

There is only one previous isolation record of this fungus colonising roots when (Andrews and Hecht 1982) reported it as being a root endophyte of Eurasian water foil (*Myriophyllum spicatum* L.).

3. *Acremonium fusidioides* (Nicot) W. Gams

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris*

This species is described as being a widespread, but not a very common soil fungus (Domsch *et al.* 1980), which is a result confirmed in survey one. It was isolated from all hosts in survey one, however was still uncommon, with only 16 isolates being obtained. This species is previously unreported from pasture plant roots.

4. *Acremonium kiliense* Gr tz

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris*

Acremonium kiliense is a ubiquitous soil fungus (Domsch *et al.* 1980), and was isolated from all 4 pasture species sampled in survey one and two. There is only one report of this fungus from roots, when it was described as a beneficial endophyte of tomato roots reducing *Fusarium* wilt symptoms (Bargmann and Schonbeck 1992). It has also been implicated in stem and basal rots of sugarcane (Sattar and Ali 1981), maize (Ivashchenko 1989), barley (Mehiar *et al.* 1976) and rice (Bessi and Carolis 1974).

5. *Acremonium murorum* (Corda) W.Gams

Isolation site: Whatawhata

Host: *Anthoxanthum odoratum; Agrostis capillaris.*

Unlike the four hyaline *Acremonium* species identified in this study, this species has dematiaceous hyphae and spores and was formerly placed in the genus *Gliomastix.* It was isolated with low frequency (5 isolates) from sweet vernal and browntop. *Acremonium murorum* is reported to be a common soil saprophyte with a worldwide distribution (Domsch *et al.* 1980, Gams 1992), and has been found in rhizospheres of wheat, barley, lucerne and in pasture soils (Domsch *et al.* 1980). It is previously unreported from either sweet vernal or browntop which are therefore new host records.

6. *Acremonium strictum* W. Gams

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum.*

Ten isolates of *A. strictum* were obtained from white clover, perennial ryegrass and sweet vernal. This species was found at both sample sites, two isolates from Whatawhata and eight from Ruakura. It was found to be highly pathogenic to seedlings of subterranean clover, red clover, white clover, sweet vernal, cocksfoot timothy, browntop and Yorkshire fog, but was non pathogenic on ryegrass, tall fescue and soft brome seedlings. Falloon (1985) also reported a species of *Acremonium* to be non pathogenic on perennial ryegrass seedlings while Skipp and Christensen (1989) reported one out of three *Acremonium* isolates tested, was pathogenic to ryegrass seedlings, the other two isolates being non pathogenic.

7. *Alternaria alternata* (Fr.) Keissler.
Isolation site: Whatawhata; Ruakura
Host: *Trifolium repens; Lolium perenne; Agrostis capillaris; Lotus uliginosus* .

This common Hyphomycete was isolated from four species; lotus, browntop, white clover and ryegrass, and was present at both Ruakura and Whatawhata pasture sites at low frequencies. This is a ubiquitous fungus found on many substrates and has been commonly isolated from the rhizospheres of many species (Domsch *et al.* 1980). There are several overseas reports of this fungus being isolated from pasture plant roots, but although widespread in distribution, it is isolated in relatively low numbers from roots (Sprague 1959, Domsch *et al.* 1980, Farr *et al.* 1989). Falloon (1985) also obtained *Alternaria* from perennial ryegrass roots.

8. *Amorphotheca resinae* Parbery
Isolation site: Ruakura
Host: *Lolium perenne*

A single isolate of this ascomycete was obtained in survey one from ryegrass. It is the teleomorph stage of *Cladosporium,* which was more commonly isolated from pasture roots. The *Cladosporium* anamorph is often reported from soils and rhizospheres (see entry 26 below) but the teleomorph has not previously been reported from pasture roots until now.

9. *Aphanocladium album* (Preuss) W. Gams
Isolation site: Whatawhata
Host: *Lolium perenne; Anthoxanthum odoratum.*

This genus was created by Gams (1971a) when the classification of *Cephalosporium* was revised. While most species of *Cephalosporium* were reclassified into *Acremonium,* several species with characteristic reduced phialides (aphanophialides) were placed into the new *Aphanocladium* genus. *Aphanocladium album* was only isolated from Whatawhata

pasture plots from perennial ryegrass (3 isolates) and sweet vernal (5 isolates), and these are new host records. There are no previous reports of this fungus from roots of pasture. There are many reports of this fungus as a hyperparasite of other fungi and insects, and has been investigated as a potential biocontrol of rusts and mildews (Mitov and Ibrakhim 1977).

10. *Arthrinium arundinis* (Corda) Dyko and B. Sutton

Isolation site: Whatawhata

Host: *Trifolium repens; Anthoxanthum odoratum; Agrostis capillaris; Bromus hordeaceus*

Arthrinium, and its teleomorph *Apiospora montagnei* Sacc., are common saprophytes of many substrates (Domsch *et al.* 1980). In Europe, *Arthrinium* species have been reported from the root regions of graminaceous hosts such as wheat and barley, as well as common grasses. It is previously unreported in New Zealand from either roots or rhizosphere. *Arthrinium arundinis* was found to be a mild pathogen of white clover and timothy, and a weak pathogen of red clover, cocksfoot, Yorkshire fog, browntop, sweet vernal and subterranean clover. It actively invaded the epidermis and cortex of the roots of these hosts seedlings.

11. *Arthrobotrys oligospora* Fres.

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Agrostis capillaris; Trifolium subterraneum.*

Arthrobotrys oligospora is the commonest and most cosmopolitan of nematode-trapping fungi (Domsch *et al.* 1980). The fungus captures nematodes by means of an adhesive network of conidiophores and sympodially formed pyriform conidia. It has a worldwide occurrence and has been reported from New Zealand soils, tussock grasslands and pastures (Wood 1973, Hay pers. comm.). There are reports of this fungus being isolated from rhizospheres of barley, soya beans, sugar beet, beans and citrus (Domsch *et al.* 1980), and there have been trials to assess its potential use for biological control of nematodes.

***Aspergillus* Mich.: Fr.**

Four species of *Aspergillus* were isolated from both Whatawhata and Ruakura (see 12-15 below). A total of 33 isolates were obtained from all three surveys. This genus commonly occurs in soil, but is less frequently isolated, than the penicillia, from soil in temperate climates (Raper and Fennell 1965). The genus can be characterised by the presence of erect conidiophores which terminate in a vesicle covered with whorls of phialides. *Aspergillus* species have been previously reported as being mild pasture root pathogens of ryegrass (*Lolium perenne, Lolium multiflorum* Lam.) seedlings (Falloon,

1985a), and have also been isolated from the root segments of cocksfoot (*Dactylis glomerata* L.), meadowgrass (*Poa pratensis* L.) and perennial ryegrass from English pastures (Gadgil 1965). Species of *Aspergillus,* and its teleomorph *Emericella* Berk., were also isolated from wheat and ryegrass (*Lolium rigidum* L.) roots in Western Australia (Dewan and Sivasithamparam 1988a). They reported *A. terreus* (Thom) was pathogenic to seedlings of both hosts, causing root rot and root weight reduction to both. This fungus was also antagonistic to the serious wheat pathogen *Gaeumannomyces graminis* .var. *tritici* Arx and D.L. Oliver. *Aspergillus flavus* Link and *A. terreus* have been isolated from roots of white clover in the USA (Farr *et al.*, 1989).

12. *Aspergillus fumigatus* Fres.
Isolation site: Whatawhata; Ruakura
Host: *Trifolium repens; Agrostis capillaris;*

Aspergillus fumigatus is a thermotolerant fungus with a worldwide distribution and has a high competitive saprophytic ability (Johri *et al.* 1975). In rhizosphere studies it has been isolated from clover, wheat, flax, rice, strawberry, pines, coffee, groundnut, barley, ferns, corn and oats (Domsch *et al.* 1980). It has not been identified from rhizosphere or root studies in New Zealand until now. *Aspergillus fumigatus* was isolated from the roots of wheat and perennial ryegrass in Western Australia (Dewan and Sivasithamparam 1988a), and was found to be non-pathogenic to both hosts. It is also a cause of systemic human disease and ranks as a major pathogen of animals, particularly birds (Raper and Fennell 1965)

13. *Aspergillus glaucus* Link ex Gray
Isolation site: Ruakura
Host: *Lolium perenne*

One isolate of this fungus was recovered in survey one from perennial ryegrass. There are no previous reports of it being associated with plant roots so this isolation is a new host record.

14. *Aspergillus niger* van Teighem
Isolation site: Whatawhata; Ruakura.
Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Holcus lanatus; Lotus uliginosus ; Trifolium subterraneum.*

Aspergillus niger was the most commonly identified species from this genus. It was isolated 26 times from six hosts, and although it penetrated the epidermal cells of tested pasture seedlings, it was found to be non-pathogenic to all tested hosts. This fungus is ubiquitous in soil and is more common in temperate soils than other Aspergilli (Domsch *et*

al. 1980). In New Zealand it has been frequently isolated from garlic (Pennycook 1989), and is reported to be inhibited by other soil and root-colonising fungi such as; *Chaetomium globosum, Fusarium oxysporum* and *Trichoderma polysporum.*

15. *Aspergillus ustus* (Bain) Thom and Church

Isolation site: Whatawhata; Ruakura.

Host: *Lolium perenne; Bromus hordeaceus*

Aspergillus ustus is a common soil-borne *Aspergillus* species (Raper and Fennell 1965), and a worldwide distribution is documented, however its main reported occurrence is from the tropics. It has rarely been identified from roots of plants, but has been reported from the rhizospheres of; lupins, wheat, corn, tomatoes and poplar. It has not previously been reported from plants roots in New Zealand and is a new host record for both hosts from which it was isolated.

16. *Aureobasidium pullulans* (de Bary) Arnaud

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum.*

Aureobasidium pullulans was isolated from sampled plant roots with a total of 27 isolates being obtained, mainly from white clover. Two varieties of this species can be distinguished on PDA. These colonies were initially pinkish, but went brown to black as the culture aged. This pigment change suggests these root isolates were *A. pullulans* var. *melanogenum,* which in culture is characteristically olivaceous to black and contrasts to *A. pullulans* var. *pullulans*, which remains cream or hyaline. This species is closely related to mycelial yeasts which are common in soil and is described as a cosmopolitan saprophyte (Domsch *et al.* 1980), being frequently isolated from leaves, especially in temperate zones. It was previously found on clover and ryegrass leaves in Waikato pastures (di Menna and Parle 1970). It is not regarded as being common on roots, as it is rarely reported from root mycofloras, however, it has been reported from the rhizospheres of grasses, wheat, corn and various sand dune plants (Domsch *et al.* 1980). A related species, *A. caulivorum* (Kirchener) W.B. Cooke has been reported as a pathogen of red clover pastures in New Zealand (Blair 1973). *Aureobasidium pullulans* has previously been isolated from white clover and red clover in the USA (Farr *et al.* 1989).

17. *Beauveria bassiana* (Bals.) Vuil.
Isolation site: Whatawhata
Host: *Trifolium repens*

One isolate of this insect pathogen was obtained from white clover, and it has previously been recorded from white clover roots (Skipp and Christensen 1982), as well as from New Zealand pasture soils (Jackson, 1965). *Beauveria bassiana* is rarely reported from plant roots and most isolation records are from insects or soil.

18. *Bimuria novae-zelandiae* D. Hawksworth, Yen and Sheridan
Isolation site: Whatawhata; Ruakura
Host: *Trifolium repens*

White clover was the only host from which *B. novae-zelandiae* was isolated at both sample sites, and was one of the few host specific species found. It was very common on clover with 81 isolates being obtained in surveys one and two. Despite being host specific to white clover, further *in vitro* pathogenicity tests showed this fungus was able to invade roots of lotus, red clover and subterranean clover, without causing disease symptoms. Penetration of grass roots was absent, however it has previously been shown to invade host root tissue of perennial ryegrass (Christensen *et al.* 1988) and it was also reported to be pathogenic to white clover seedlings causing necrotic lesions to rot tissues. *Bimuria novae-zelandiae* was first isolated from soil under barley crops in Wairarapa (Hawksworth *et al.* 1979) and has only been reported from New Zealand. Since its original description it has been frequently isolated from white clover roots (Sarathchandra *et al.* 1995, Skipp and Christensen 1981, Skipp and Christensen 1982, Skipp and Christensen 1983) and from red clover (Skipp *et al.* 1986). This ascomycete has a remarkable morphology producing two large brown ascospores in a single ascus and ascomata were copiously produced on seedling roots of clovers. This indigenous asomycete is the taxonomic order Dothideales, in the family Pleosporaceae, characterised by their black, thick-walled, ostiolate ascomata and brown, septate and muriform ascospores.

19. *Bipolaris*
Isolation site: Whatawhata
Host: *Trifolium repens*

One isolate of *Bipolaris* was obtained in survey one from white clover. *Bipolaris* is unrecorded on pasture roots in New Zealand but its teleomorph, *Cochliobolus,* has been reported from the leaves of pasture species such as ryegrass and cocksfoot (Pennycook 1989). *Bipolaris sorokiniana* Sacc. and its teleomorph *C. sativus* Ito and Karub. are reported to be serious root rot pathogens of a wide range of grass and cereal hosts worldwide (Farr *et al.* 1989, Stack 1992).

20. *Botryosporium*
Isolation site: Ruakura
Host: *Trifolium repens*

Like *Bipolaris*, one isolate of *Botryosporium* was recorded from white clover which is a new host record as reports of this genus from roots are rare. Some species are reported to be pathogenic to tobacco plant roots (Anderson and Welacky 1983).

21. *Botrytis cinerea* Pers. : Fr.
Isolation site: Whatawhata; Ruakura
Host: *Trifolium repens; Lolium perenne; Agrostis capillaris; Holcus lanatus.*

Although this fungus was found at both sites in all three surveys, it was relatively rare with only nine isolates being recorded. It is a widespread and virulent plant pathogen with a broad host range (Pennycook 1989). This was further demonstrated in pathogenicity tests where it invaded the roots of pasture seedlings, killing all white clover, red clover and browntop seedlings and causing root rot lesions on; perennial ryegrass, subterranean clover, lotus, soft brome, tall fescue, timothy, Yorkshire fog, sweet vernal and cocksfoot seedlings. *Botrytis cinerea* has been previously reported in the epidermis and cortex of roots of ryegrass seedlings (Sprague 1950).

***Chaetomium* Kunze ex Fr**

This was the second most frequently recorded ascomycete genus isolated in the study. *Chaetomium* is commonly reported from soils, and has been reported in New Zealand from roots of red clover (Skipp *et al.* 1986), white clover (Skipp and Christensen 1982) and maize (Falloon 1982, Fowler 1985). Gadgil (1965) also reported this genus to be common on roots of perennial ryegrass, meadowgrass and cocksfoot. This genus is classed in the ascomycete order Sphaeriales in the family Chaetomiaceae.

22. *Chaetomium funicola* Kunze ex Steud.
Isolation site: Whatawhata; Ruakura
Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris.*

Chaetomium funicola was the commonest species of this genus to be isolated, 26 isolates being obtained. It invaded epidermal tissues of, perennial ryegrass, white clover, subterranean clover, soft brome, lotus, tall fescue, timothy and browntop, but was non-pathogenic.

23. *Chaetomium globosum* Cooke

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Bromus hordeaceus.*

Chaetomium globosum was isolated at both sites from all hosts in survey one and from soft brome in survey three. Seedling roots of; perennial ryegrass, white clover, subterranean clover, red clover, soft brome, lotus, tall fescue, timothy, sweet vernal and Yorkshire fog, were recolonised in *in vitro* tests but like *C. funicola*, it was non-pathogenic to seedlings. This result contrasted with Falloon (1985), who isolated this fungus from perennial and Italian ryegrass seedling roots, and found it to be pathogenic to seedlings.

24. *Chaetomium indicum* Corda

Isolation site: Whatawhata

Host: *Lolium perenne.*

Only one isolate of *C. indicum* was recorded in all surveys. It is unreported from pasture or roots in New Zealand and is a new host record.

25. *Chrysosporium* Corda

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne.*

Seven isolates were recorded from roots, six from clover and one from ryegrass. Species of *Chrysosporium* have been previously isolated from red clover roots (Skipp *et al.* 1986), and from white clover roots at over 48 pasture sites throughout New Zealand (Skipp and Christensen 1981, Skipp and Christensen 1982, Skipp and Christensen 1983, Skipp and Watson 1987) and was reported to be present on the rhizoplane as well as roots of clover. This species appeared to be host specific to white clover under field conditions and was pathogenic to white clover seedlings (Skipp and Christensen 1982, Skipp and Watson 1987). Overseas studies have recorded different species of *Chrysosporium* from the roots of potato (Dashwood *et al.* 1993), peas and other legumes (Grosch *et al.* 1995), and high altitude pasture soils in Czeck (Palencarova 1977) and Canada (Bissett and Parkinson 1979).

26. *Cladosporium herbarum* (Pers.) Link ex Gray

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Lotus uliginosus* .

Cladosporium herbarum was common at both sites with 34 isolates being identified, and was isolated from all hosts in surveys one and two. In survey three an additional isolate was recorded from *lotus*. This fungus is generally regarded to be a saprophyte of plants and has been frequently recorded from pasture leaves or plant debris (di Menna and Parle 1970, Jackson 1965, McKenzie 1971). Waid (1957) also reported this fungus to colonise the surface and cortex root tissues of perennial ryegrass.

27. *Clasterosporium* Schweinitz

Isolation site: Whatawhata; Ruakura

Host: *Lolium perenne; Anthoxanthum odoratum*

Eight isolates of this hyphomycete were identified from roots, one from sweet vernal and seven isolates from perennial ryegrass (five from Ruakura and one from Whatawhata). This fungus is unreported from pasture roots and is a new host record for sweet vernal. This root-colonising hyphomycete was found to invade epidermal root tissue of all tested grass and legume hosts (red clover, white clover, subterranean clover, perennial ryegrass, cocksfoot, Yorkshire fog, lotus, tall fescue, timothy, browntop, sweet vernal and soft brome). In pathogenicity tests, *Clasterosporium*, was found to be pathogenic to red clover, white clover, subterranean clover, perennial ryegrass and cocksfoot, causing root rot lesions and seedling death.

28. *Codinaea fertilis* Hughes and Kendrick (= *Dictyochaeta fertilis*)

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus; Lotus uliginosus ; Trifolium subterranean.*

Codinaea fertilis was ubiquitous on pasture plant roots in all three surveys. It was the second most common species identified in surveys one and two with 658 isolates (9.5%) being recorded. Although it was isolated from the three grass hosts (perennial ryegrass 177 isolates, 78 from Whatawhata and 99 from Ruakura; sweet vernal, 56 isolates; browntop, 62 isolates) it was most frequently isolated from white clover, with 363 isolates being obtained, 134 from Whatawhata and 229 from Ruakura. In survey three, *C. fertilis* was the most common fungus isolated, comprising 15.3% of the total 333 fungi obtained. Although it was absent from soft brome roots, it was present on Yorkshire fog (eight isolates), lotus (20 isolates) and subterranean clover (23 isolates). In assessments of *in vitro* root-colonisation of host seedlings, *C. fertilis* invaded the epidermis of; ryegrass,

subterranean clover, white clover, red clover, lotus, tall fescue, sweet vernal and Yorkshire fog, the cortex of; timothy and cocksfoot and the vascular tissue and inner cortex of browntop. Lim and Cole (1984) also reported this fungus to infect epidermis and cortex tissue of white clover. It did not invade any root tissues of soft brome seedlings, which may explain its absence from this species in survey two. Mean disease scores classed this fungus as pathogenic to; white clover, browntop, red clover and timothy (100% mortality), mildly pathogenic to; ryegrass, sweet vernal, Yorkshire fog and lotus, and non-pathogenic to; soft brome, tall fescue, subterranean clover, and cocksfoot .

Other surveys of root-colonising fungi in New Zealand pastures have also found this fungus to be ubiquitous on roots (Pennycook 1989) however, it has rarely been recorded from overseas studies with an exception of the USA where Campbell (1980, 1982) isolated it from white clover in North Carolina. It has been shown to be a serious pathogen of white clover and other forage legumes affecting productivity and persistence in pastures (Campbell 1980, Campbell 1982, Menzies 1973a, Skipp and Christensen 1982).

29. *Colletotrichum* Corda

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris.*

This genus belongs to the class Ceolomycetes, which also includes the pycnidial fungi (see separate entry). On Hay agar, *Colletotrichum* readily produced conidia in acervuli surrounded by dark sterile setae allowing identification. There were two species of *Colletotrichum* which were obtained in the study, 25 isolates were similar to the description of *C. dematium* (Pers. ex Fr.) Grove. The remaining two isolates were obtained from perennial ryegrass and were morphologically similar to *C. graminicola* (Ces.) G.W. Wils, however, further morphological study is needed to confirm both preliminary identifications. Of the total of 27 isolates obtained, most were isolated from white clover . From *in vitro* seedling tests it was found to be pathogenic to red, white and subterranean clovers, lotus and browntop, while being mildly pathogenic to; ryegrass, Yorkshire fog, cocksfoot, and non-pathogenic to sweet vernal, timothy, soft brome and tall fescue. Species of *Colletotrichum* have been previously reported from the roots of red clover (Nan *et al.* 1991b) and white clover (Skipp and Christensen 1982, 1983) *Colletotrichum* is also a common and widespread leaf pathogen of pasture grasses in New Zealand (McKenzie and Latch 1984, Pennycook 1989).

30. *Curvularia trifolii* (Kauffm) Boedijn

Isolation site: Whatawhata

Host: *Lolium perenne; Agrostis capillaris.*

Three isolates of *C. trifolii* were identified from perennial ryegrass (two isolates) and browntop (one isolate) at Whatawhata. *In vitro* seedling tests confirmed it as a root-colonising fungus, as it was able to recolonise seedling roots of all tested hosts. These tests demonstrated that it was a root pathogen of red and white clover, and a weak pathogen of subterranean clover and lotus. It was classed as non-pathogenic to all remaining grass hosts. It has also been previously isolated from perennial and Italian ryegrass roots (Falloon 1985), however, it is more commonly reported as a leaf pathogen of grasses including browntop, cocksfoot and *Poa annua* L. (Falloon 1976, McKenzie 1978).

31. *Cylindrocarpon destructans* (Zins.) Scholten (= *Cylindrocarpon radicola* Wollenw.; teleomorph = *Nectria radicola* Girlach and Nilsson)

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris.*

This fungus was very common in surveys one and two, as a total of 139 isolates (2%) were recorded from all four hosts at both sites. It was found to re-invade root tissues of all inoculated seedlings, and was pathogenic to; red clover, white clover; subterranean clover browntop and ryegrass, mildly pathogenic to; Yorkshire fog, lotus, sweet vernal, and timothy, and non-pathogenic to; soft brome, tall fescue and cocksfoot. *Cylindrocarpon destructans* is ubiquitous on roots of many species, and in New Zealand it has been reported on the pasture roots of red clover (Skipp *et al.* 1986, Nan *et al.* 1991a, 1991b), white clover (Skipp and Christensen 1981, 1982, Sarathchandra *et al.* 1995) and perennial ryegrass (Thornton 1965, Falloon 1985, Skipp and Christensen 1989, Bonish unpublished). It has a worldwide occurrence and has been frequently reported from plant roots (Brayford 1992; Taylor and Parkinson 1964). *Cylindrocarpon* spp. have been reported to be more abundant in alkaline soils than acidic soils (Matturi and Stenton 1964), who also reported *C. destructans* as being abundant and competitively saprophytic on strawberry roots. This species is an abundant and primary colonizer of plant roots (Stenton 1958), and it has been reported as both a pathogen and saprophyte of plant roots. The distinction between different strains of *C. destructans* which are root pathogens and cause disease symptoms, opportunistic pathogenic stains which cause disease only under specific environmental conditions, or saprophytic strains that have colonised living tissues, is not always apparent, and is probably why there is some confusion over its pathogenicity status in the literature (Matturi and Stenton 1964, Brayford 1992).

32. *Cylindrocladium scoparium* Morgan

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Trifolium subterranean.*

Cylindrocladium scoparium was frequently isolated from white clover roots from which 85 isolates were recorded (31 isolates from Whatawhata, 54 isolates from Ruakura). It was rarely encountered on the remaining hosts, but was isolated from perennial ryegrass (six isolates) and subterranean clover (one isolate in survey three). It was not host specific to white clover as root tissue of seedlings of; browntop, red clover, timothy; ryegrass, sweet vernal, Yorkshire fog, lotus, soft brome, tall fescue, subterranean clover and cocksfoot were also re-invaded causing root rot and high mortality to all hosts. This fungus is a virulent and widespread root rot pathogen of many plants species (Pennycook 1989, Hunter 1992), and has a worldwide occurrence. It has been previously isolated in New Zealand pastures from red clover roots (Skipp *et al.* 1986, Nan *et al.* 1991b), and has also been reported from white clover and perennial ryegrass seedlings (Sarathchandra *et al.* 1995, Bonish unpublished). Freter and Wilcoxson (1964) also recorded this fungus as a root pathogen from pastures of red clover, lucerne (*Medicago sativa* L.) and sweet clover (*Melilotus* spp.). *Cylindrocladium scoparium* has additionally been reported as a leaf-spot and stem-blight pathogen of lucerne pastures, particularly in wet weather (Ponappa *et al.*, 1977). The majority of isolation records of this fungus in New Zealand and the world, have been as pathogens of many tree species, particularly tree seedlings (Hunter 1992, Pennycook 1989). *Cylindrocladium scoparium* is an obligate plant parasite and only persists in soil by producing microsclerotia and conidia.

33. *Dactylaria acerosa* Mats

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus; Lotus uliginosus ; Bromus hordeaceus.*

Dactylaria acerosa is a slow growing hyaline hyphomycete which required very nutrient poor media such as Hay agar or PCA with incubation under near-UV light to sporulate. Conidia were filiform and septate and produced on small denticles. It was frequently isolated from all species at Whatawhata in survey one, with 67 of the 71 isolates being found at Whatawhata plots. The remaining four isolates were obtained in survey two from white clover roots at Ruakura. In survey three it was isolated from Yorkshire fog (one isolate), lotus (one isolate) and soft brome (two isolates). These are all new host records as this species has not been previously reported from plant roots, although Skipp and Christensen (1989) isolated a species of this genus from perennial ryegrass roots. *Dactylaria acerosa* was found to be non-pathogenic to all 12 pasture species tested but was found to be root-invading, as the epidermis of ; perennial ryegrass, white clover, subterranean clover, red clover, lotus, tall fescue, timothy, browntop, sweet vernal and

Yorkshire fog were recolonised by hyphae. Roots of soft brome and cocksfoot were absent of hyphal colonisation. Overseas studies report species of *Dactylaria* as nematode-trapping fungi, as well as colonising the roots or rhizospheres of plants such as cotton (Shagalina *et al.* 1971) and lucerne (Rama Rao and Rao 1973).

34. *Dreschlera dematioidea* Bubak and Wroblenski

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris.*

Fifteen isolates of *D. dematioidea* were recorded and although uncommon, this species was isolated from ryegrass at Ruakura (one isolate), and from clover (two isolates), sweet vernal (six isolates), browntop (three isolates) at Whatawhata. It was observed to systemically colonise seedling roots and was pathogenic to; red clover, white clover, subterranean clover, ryegrass, Yorkshire fog, cocksfoot, browntop, timothy and soft brome, and mildly pathogenic to; lotus, sweet vernal and tall fescue. Previous isolation reports show it is commonly found on grass leaves (McKenzie 1978, Pennycook 1989), being recorded on sweet vernal, ryegrasses, fescues, timothy, browntop and cocksfoot. Reports from roots are rare, however, Labruyere (1979), recorded an isolate from ryegrass roots in Dutch pastures. Falloon (1985), reported the closely related species, *Dreschlera siccans* Dreschler, was non pathogenic to roots of ryegrass seedlings.

35. *Epicoccum nigrum* Link (= *Epicoccum purpurascens* Ehrenberg)

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus.*

Regarded as saprophyte, *E. nigrum,* has worldwide and widespread distribution, and is isolated in numerous mycological surveys. This study was no exception with fifteen isolates being obtained all four hosts in the first two surveys, and one isolate from Yorkshire fog in survey three.

Fusarium Link ex Fr.

Fusarium is one of the most economically important and soil borne genera and is pathogenic to most agricultural, horticultural and silvicultural crops grown in the world (Windels, 1992). It is pathogenic to important pasture species (Burgess *et al.* 1988, Farr *et al.* 1989, Kilpatrick and Dunn 1961, Menzies 1973b, Shipton 1967, Skipp *et al.* 1986; Sprague 1950, Wong *et al.*, 1984), and several species are also virulent pathogens of pasture seed and seedlings (Falloon 1985a, Holmes 1976, Holmes 1983, McGee and Kellock 1974, Skipp and Christensen 1982, Skipp and Christensen 1989). Soil borne *Fusarium* species persist as chlamydospores, or as hyphae in plant debris (Burgess 1981, Jackson 1965), and species, such as *F. oxysporum* and *F. culmorum,* can exist as soil saprophytes (Nelson *et al.* 1986, Gams 1992). Many species of *Fusaria* are also important

secondary colonisers of old or dying plant roots (Waid 1957, Burgess *et al.* 1988). They can also exist as aggressive primary root invaders causing root rots (Burgess *et al.* 1988, Leath and Kendall 1978, Nelson *et al.* 1986, Sprague 1950) . Morphologically these pathogenic and saprophytic populations are indistinguishable, thus pathogenicity tests are required to determine which isolates are pathogens. Several fusaria are often isolated together from root rot lesions and so are referred to as a "root rot complex" (Sprague 1950, Shipton 1967, Kommedahl and Windels 1979), as it is unclear which are primary and secondary invaders, but the end result is still serious root rot damage to plant hosts. *Fusarium* root pathogens can also cause wilt disease of pasture plants by infecting roots and plugging the xylem vessels (Pratt 1982, Venuto *et al.* 1995).

In previous surveys of fungi invading pasture roots in New Zealand, *Fusarium* was one of the commonest genera isolated (Thornton 1965, Skipp and Christensen 1982, 1989, Skipp *et al* 1986, Nan *et al* 1991b, Bonish unpublished). Ten *Fusarium* species (see 36-45 below) were isolated from the sampled sites and was the commonest genus recorded in all three surveys (see tables 2 and 3).

Table 2. *Fusarium* species isolated from pasture plants in survey one and two.

Species	Ryegrass *	Clover *	Ryegrass	Clover	Browntop	Sweet Vernal	**Total**	**%**
F. acuminatum	2	3	4	2	2	1	**14**	**1.3**
F. avenaceum	25	31	1	27	2	9	**95**	**8.7**
F.crookwellense	28	35	12	14	7	6	**102**	**9.3**
F. culmorum	47	10	4	3	1	1	**66**	**6.0**
F. equiseti	-	-	1	-	-	-	**1**	**<1**
F. graminum	-	-	-	-	-	2	**2**	**<1**
F. oxysporum	136	247	102	147	98	42	**772**	**70.4**
F. sambucinum	-	-	-	-	-	1	**1**	**<1**
F. solani	12	17	5	2	4	1	**41**	**3.7**
F. tricinctum	-	-	-	-	-	2	**2**	**<1**
TOTAL	250	343	129	195	114	65	**1096**	**100**

* Ryegrass and clover sampled from improved dairy pasture Ruakura, the remaining species sampled from unimproved hill pasture at Whatawhata

A total of 66 *Fusarium* isolates were obtained in survey three (table 3) and this comprised 19.8 % of all fungi identified in this survey.

Table 3. *Fusarium* species isolated from pasture plants in survey three

SPECIES	Subterranean clover	Lotus	Yorkshire fog	Soft brome	Total	%
F. avenaceum	5	-	1	1	**7**	**10.5**
F. crookwellense	1	1	-	-	**2**	**3.0**
F. culmorum	-	5	2	-	**7**	**10.5**
F. equiseti	-	-	-	1	**1**	**1.5**
F. oxysporum	7	19	14	6	**46**	**70**
F. tricinctum	2	-	1	-	**3**	**4.5**
Total	**15**	**25**	**18**	**8**	**66**	**100**

Other species of this genus that have previously been isolated from pasture roots include, *F. anthophilum* (Sarathchandra *et al.* 1995), *F. nivale* (Skipp and Christensen 1982), and *F. poae* (Pennycook 1989).

***In vitro* root-colonisation and pathogenicity to seedlings.**

All isolates screened, produced visible symptoms of root disease in all hosts. *Fusarium acuminatum*, *F. avenaceum, F. culmorum, F. crookwellense* and *F. tricinctum* were highly pathogenic to all hosts, resulting in severe root rot and seedling death (table 4), and all had high virulence scores. *Fusarium graminum* caused lesioning and chlorosis of roots on clover hosts, but was avirulent to tested grass hosts. *Fusarium equiseti, F. oxysporum, F. sambucinum,* and *F. solani,* were variable in pathogenicity to the different hosts ranging from being weakly pathogenic to highly pathogenic, depending on host species. All leguminous seedlings were more susceptible to *Fusarium* root rot than grass seedlings, most having higher virulence scores (Table 4).

Table 4. Virulence rating of *Fusarium* species on pasture hosts.

Species	WHITE CLOVER	SUB CLOVER	RED CLOVER	LOTUS	RYE GRASS	TALL FESCUE	GOOSE GRASS	TIMOTHY	SWEET VERNAL	BROWN TOP	COCKS FOOT	Yorkshire FOG	Average
F.acuminatum	4.33	4.50	5.00	4.83	4.83	4.17	4.50	4.67	3.83	5.00	4.67	4.67	**4.58**
F.avenaceum	5.00	5.00	5.00	5.00	5.00	4.83	4.67	5.00	4.83	5.00	5.00	5.00	**4.94**
F.crookwell.	5.00	4.67	5.00	5.00	4.83	4.17	2.83	5.00	5.00	5.00	4.83	5.00	**4.57**
F.culmorum	5.00	4.50	4.33	4.83	4.50	4.00	2.50	5.00	3.00	5.00	5.00	4.67	**4.36**
F.equiseti	4.00	3.33	4.67	3.33	2.50	2.83	3.00	2.33	3.33	2.50	3.33	2.50	**3.14**
F.graminum	3.33	3.33	3.67	2.17	1.67	0.33	0.67	2.33	1.33	0.67	2.67	3.33	**2.13**
F.oxysporum	5.00	4.67	3.50	4.50	2.00	1.83	1.00	5.00	3.67	4.33	4.33	4.00	**3.51**
F.sambucinum	5.00	4.50	2.67	4.17	4.50	2.33	3.00	3.00	2.00	3.33	3.50	4.50	**3.54**
F.solani	4.67	4.00	3.00	5.00	3.17	2.50	1.67	2.17	2.00	2.33	3.00	3.83	**3.11**
F.tricinctum	5.00	5.00	5.00	5.00	4.50	5.00	2.50	5.00	5.00	5.00	5.00	5.00	**4.75**
Control	0.33	0.00	0.22	0.11	0.33	0.00	0.00	0.00	0.22	0.33	0.50	0.22	**0.19**
Average*	**4.63**	**4.35**	**4.18**	**4.38**	**3.75**	**3.20**	**2.63**	**3.95**	**3.40**	**3.82**	**4.13**	**4.25**	

* Average score for each host species does not include control scores.

All isolates were able to penetrate and colonise epidermal and cortex tissue seedling roots (table 5). Sporodochia were often copiously produced on epidermal tissues, and chlamydospores of *F. oxysporum* were observed in root cells of lotus, subterranean clover and ryegrass. *Fusarium graminum* was not observed to penetrate any tissue of soft brome or tall fescue. Hyphae of *F. equiseti* were observed only in the epidermis of most roots, except where it invaded the cortex of cocksfoot. All hyphae of fusaria which colonised soft brome roots were restricted to the epidermis, except *F. crookwellense* which also colonised the cortex. Leguminous hosts had higher hyphal penetration scores as vascular and cortex tissue were often colonised.

Table 5. Root colonisation of pasture seedlings by *Fusarium* species.

Species	WHITE CLOVER	SUB CLOVER	RED CLOVER	LOTUS	RYE GRASS	TALL FESCUE	GOOSE GRASS	Timothy	SWEET VERNAL	BROWN TOP	COCKS FOOT	Yorkshire Fog	**Average**
F.acuminatum	3	3	2	2	2	2	1	3	3	2	2	1	**2.2**
F.avenaceum	2	3	3	2	3	2	1	3	3	3	3	3	**2.6**
F.crookwell.	2	3	1	3	3	2	2	3	3	3	3	3	**2.6**
F.culmorum	3	3	3	2	3	3	1	3	3	3	3	3	**2.8**
F.equiseti	1	1	1	1	1	1	1	1	1	1	2	1	**0.9**
F.graminum	2	3	1	2	2	0	0	2	1	2	2	2	**1.6**
F.oxysporum	2	2	2	2	2	2	1	3	3	3	3	3	**2.3**
F.sambucinum	1	3	3	2	2	1	1	3	2	3	3	3	**2.3**
F.solani	1	2	1	2	2	2	1	2	2	3	1	1	**1.7**
F.tricinctum	3	3	3	2	2	3	1	3	2	3	3	3	**2.6**
Average	**2.1**	**2.6**	**2.0**	**2.0**	**2.2**	**1.8**	**0.9**	**2.6**	**2.3**	**2.6**	**2.5**	**2.3**	

36. *Fusarium acuminatum* Ell. and Ev.

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris;*

This species has rarely been recorded from pasture roots in New Zealand but it is common in temperate grasslands and cultivated soils in Australia (Burgess *et al.* 1988). It was also the most frequently isolated *Fusarium* from the roots of pasture grasses in Iceland (Kommedahl and Windels, 1979). It is generally regarded as a saprophyte or secondary coloniser of necrotic root tissues but has been reported to cause severe root rot on clover species (Burgess *et al.* 1988). This species is reported in the USA as a causal agent of root rot of grasses and cereals, but is often isolated with other root rotting fungi (Sprague 1950, Sprague 1959), and is a common saprophyte on most graminae in the western USA, and a weak parasite of wheat and barley. Farr *et al.* (1989), also reported this species to cause

secondary root rot of many pasture species in the USA including; browntop, tall fescue, timothy, ryegrass, soft brome, cocksfoot and meadowgrass.

37. *Fusarium avenaceum* (Fr.) Sacc.

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus; Trifolium subterraneum; Bromus hordeaceus.*

There are numerous reports of *F. avenaceum* on grass and clover roots (Pennycook 1989, Farr *et al.* 1989), and it is recognised root rot pathogen of pasture legumes (Burgess *et al.* 1988, Chi *et al.* 1964, Wong *et al.* 1984). This species was found to be the most frequently isolated *Fusarium* from grass roots in Minnesota pastures (Kommedahl *et al.* 1975). Like most of the fusaria isolated in this study, it is a widespread root pathogen, and has also been isolated in New Zealand from roots of lucerne, brassicas, wheat and maize (Pennycook 1989). Intracellular and intercellular invasion of white clover roots has also been observed previously (Lim and Cole 1984). In the USA *F. avenaceum,* has been reported to cause primary root rot of browntop (Farr *et al.* 1989), *Fusarium* root rot of winter wheat (Smiley and Patterson 1996), and in Australia it has been reported to be a seed and root pathogen of subterranean clover (Shipton 1967, McGee and Kellock 1974, Wong *et al.* 1984).

38. *Fusarium crookwellense* Burgess, Nelson and Tousson

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Lotus uliginosus ; Trifolium subterraneum.*

Fusarium crookwellense was the second most frequently isolated *Fusarium* in the study with 102 isolates being obtained. It was first described from Australia in 1971 but has been recorded from a wide range of plant roots especially those in temperate grassland and temperate soils (Burgess *et al.* 1988). There is still some taxonomic debate in the literature on the naming of this species, as Nirenberg (1995), suggests this species was originally described as *Fusarium culmorum* var. *cerealis*, and therefore this name has priority over the current name. However, this has not yet been accepted by other *Fusarium* researchers. In New Zealand, it has been reported less frequently than other root-colonising fusaria, exceptions include Sarathchandra *et al.* (1995) where it was recorded on white clover and perennial ryegrass roots. Bonish and di Menna (1993) frequently isolated it from pasture herbage, and it appears to be more common in warmer and wet areas of New Zealand (di Menna pers. comm.).

39. *Fusarium culmorum* WG Smith (Sacc.)

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus; Lotus uliginosus* .

Like *F. avenaceum,* this fungus has been widely reported to cause root and crown rot of many important pasture species, particularly from forage legumes and cereals. A total of 73 isolates were identified from all three surveys. In New Zealand it has been reported from roots of perennial ryegrass (Thornton 1965, Falloon 1985, Skipp and Christensen 1989), Italian ryegrass (Falloon 1985), and white clover (Thornton 1965, Skipp and Christensen 1982, 1983, Skipp *et al.* 1986). *Fusarium culmorum* causes pre-emergence killing, stunting and brown root rot of cereals and grasses, and has been shown in inoculation trials to be a destructive parasite of important crops, such as oats and barley (Sprague 1950). It has also been reported to limit winter wheat production, as it was present in 90% of plants affected by dryland root rot in the Pacific Northwest states of Idaho, Washington and Oregon (Smiley and Patterson, 1996). In the USA it has also been isolated from the roots of the following pasture species; cocksfoot, ryegrass, tall fescue and soft brome (Farr *et al.* 1989). In a survey of Colorado grassland this fungus was the most abundant fungus encountered in the soil, rhizosphere and roots of grasses and barley (Kreutzer 1972). Waid (1974) reported *F. culmorum* dominates the root mycofloras of grasses where there is a high nitrogen content in the soil. Its success as a soilborne pathogen is probably due to its competitive saprophytic ability (Gams 1992), which enables it to exist as a common soil saprophyte in the absence of suitable plant hosts.

40. *Fusarium equiseti* (Corda) Sacc.

Isolation site: Whatawhata

Host: *Lolium perenne; Bromus hordeaceus*

Only two isolates of this cosmopolitan fungus were isolated from Whatawhata pastures, one from ryegrass in survey one and one from soft brome in survey three. It has previously been reported as being highly pathogenic to ryegrass seedlings (Falloon 1985), was very common on pasture herbage (Bonish and di Menna 1993), and has often been recorded from wheat (Pennycook 1989). Burgess *et al.* (1988) reported this fungus to be unimportant as a pathogen in Australia, which contrasts to the results in this study. However, it has been reported as a secondary root pathogen of pasture grasses and cereals (Sprague 1950, Farr *et al.* 1989), being isolated from species such as soft brome and tall fescue. This fungus was also found to be a common soil saprophyte in Dutch agricultural soils (Gams 1992).

41. *Fusarium graminum*

Isolation site: Whatawhata

Host: *Anthoxanthum odoratum*

Two isolates of *Fusarium graminum* were recorded from sweet vernal roots which is a new host record. There are few previous reports of this *Fusarium* species on pasture roots, however, Burgess *et al.* (1973) reported it being associated with root-rot of subterranean clover in Australia. It has frequently been found in association with *Claviceps paspali* Steven and Hall on inflorescences of grasses (Burgess *et al.* 1988). This study is the first report of *F. graminum* directly causing root disease of plant roots.

42. *Fusarium oxysporum* Schlecht. emend. Snyder and Hansen

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus; Lotus uliginosus ; Trifolium subterraneum; Bromus hordeaceus.*

Fusarium oxysporum was the most common species isolated in this study, comprising 9.7 % of all isolates in survey one, 13.3% in survey two and 13% in survey three. A total of 818 isolates were recorded from all three surveys. It is variable in cultural morphology and plant pathogenicity, and there are highly pathogenic strains which cause vascular wilts and root rots. Over 100 formae speciales and races have been described by plant pathologists (Burgess *et al.* 1988). Root colonising isolates are largely saprophytic but will commonly colonise necrotic roots as secondary invaders, and therefore are often mistakenly assumed to be the primary cause of root rot (Burgess *et al.* 1988). This fungus has been reported from many plant hosts in New Zealand (Pennycook 1989), and is frequently isolated from pastures (Thornton 1965, Skipp and Christensen 1982, 1983, 1989, Skipp *et al.* 1986). It is also recorded from pasture roots in overseas reports where its reported pathogenicity ranges from a saprophyte of dead pink roots or weakly pathogenic, to being a primary pathogen of pasture grasses and cereals (Sprague 1950, 1959). It has been isolated from the roots, stems and seeds of pasture legumes (Farr *et al.* 1989), where it was also reported to cause vascular wilt and root rot of white clover and red clover. In Australia where subterranean clover is a common pasture legume, *F. oxysporum* has been frequently reported to cause root rot of this important pasture plant (Barbetti and MacNish 1978, Kellock *et al.* 1978, Shipton 1967, Wong *et al.* 1984). In contrast to *F. culmorum,* which is prevalent in high fertility (fertilizer inputs) pastures, *F. oxysporum* was the most abundant fungus encountered in the soil, rhizospheres and roots of grasses grown in pastures without fertilizer inputs and in mixed pasture swards of grasses and clovers (Thornton 1965, Kreutzer 1972, Waid 1974). A similar result was found in this study, as *F. oxysporum* was the most abundant species in both low fertility Whatawhata pastures and high fertility, mixed sward (clover and ryegrass) Ruakura pastures.

43. *Fusarium sambucinum* Fuckel.

Isolation site: Whatawhata

Host: *Anthoxanthum odoratum*

Fusarium sambucinum was isolated once from sweet vernal, which is another new host record. It has been recorded from lucerne roots in New Zealand (Harvey 1982) and has been frequently reported to cause leaf spot and crown rot of lucerne (Harvey and Martin 1980, Pennycook 1989). Otherwise there are no reports of this fungus from plant roots in New Zealand. *Fusarium sambucinum* has also been isolated from necrotic roots of subterranean clover in Australia (Burgess *et al.* 1973), and it is reported to be more common in cool to cold temperate regions (Burgess *et al.* 1988).

44. *Fusarium solani* (Mart.) Appel and Wollenw. emend. Snyder and Hansen

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris.*

Fusarium solani was isolated 41 times in this study. It is a cosmopolitan soil borne fungus which is common in cultivated and grassland soils (Burgess *et al.* 1988). In New Zealand, *F. solani* has been isolated from roots of red clover (Skipp *et al.* 1986) and was found to be pathogenic to red clover roots. Falloon (1985) also reported the fungus to be pathogenic to perennial and Italian ryegrasses. Some strains of *F. solani* have been reported to cause severe crown and root rots of a wide range of plant species (Farr *et al.* 1989), including white clover. As a soilborne pathogen, *F. solani*, has been reported to reduce root nodule number as well as root weight in legume crops, such as soybean (*Glycine max* L.) and cause root necrosis (Forbes and Davet 1991). *Fusarium solani* was the most abundant fungus encountered the roots of sugar beet (*Beta vulgaris* L.), and the second most prevalent fungus on the roots of grasses in high fertility Colorado grasslands (Kreutzer 1972).

45. *Fusarium tricinctum* (Corda) Sacc.

Isolation site: Whatawhata

Host: *Anthoxanthum odoratum; Holcus lanatus; Trifolium subterraneum.*

Two isolates of *F. tricinctum* were found on sweet vernal roots in survey one, and three isolates were obtained in survey three, one from Yorkshire fog and two from subterranean clover. All five isolations are new hosts records in New Zealand. It has been isolated from pasture herbage (Bonish and di Menna 1993) especially in dry cool areas. It is rarely reported from Australian pastures and soils (Burgess *et al.* 1988). Further tests showed this fungus was a highly virulent pathogen to all grass and clover seedlings tested.

46. *Geotrichum candidum* Link ex Leman

Isolation site: Whatawhata

Host: *Anthoxanthum odoratum*

This species is a common soil borne yeast and was isolated once from sweet vernal, and this is a new host record. A species of *Geotrichum* has also been reported from red clover roots in the USA (Farr *et al.* 1989), and has been observed to infect the living hairs of wheat roots (Allan *et al.* 1992).

47. *Gliocladium roseum* Bain

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Lotus uliginosus ; Trifolium subterraneum; Bromus hordeaceus.*

Gliocladium roseum was the third most frequently identified fungus comprising 2.2% of fungi (154 isolates) in surveys one and two. It was also isolated from lotus, subterranean clover and soft brome in survey three. Roots of all grass and clover seedlings were colonised by *G. roseum*, and it was pathogenic to; white clover, subterranean clover, red clover, sweet vernal, browntop, Yorkshire fog and browntop, mildly pathogenic to; ryegrass, soft brome, tall fescue and timothy, and non pathogenic to lotus. This hyaline hyphomycete is ubiquitous in soil and plant roots, and has been widely reported in previous mycological studies on pasture roots (Sprague 1950, Waid 1957, Gadgil 1965, Thornton 1965, Skipp and Christensen 1982, 1983, 1989, Skipp *et al.* 1982, 1986). This fungus also exists as a common soil saprohyte and is frequently reported from mycological soil surveys (Jackson 1965, Bissett and Parkinson 1979, Widden 1986, Gams 1992, Dix and Webster 1995).

48. *Gliocladium* spp.

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum*

Two additional unidentified species of *Gliocladium* were isolated in surveys one and two. The first species resembled *G. catenulatum* Gilm. and Abbott as it possessed whorled phialides, and five isolates were found on ryegrass, clover and sweet vernal. A second species was isolated once from ryegrass at Whatawhata and produced very small hyaline conidia (2-4µm in diameter), resembling *Gliocladium deliquescens* Sopp, but further identification is required to confirm this. Skipp and Christensen (1982) found several species of *Gliocladium* were pathogenic to white clover seedlings and *Gliocladium*-like species have also been reported as endophytes of perennial ryegrass (Latch *et al.* 1984).

49. *Gongronella butleri* Lendner, Peyronel and Dal Vesco

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Lotus uliginosus ; Trifolium subterraneum.*

Gongronella butleri was the most common zygomycete species isolated in this study. It was ubiquitous on roots with 47 isolates being recorded, two of the isolates were from sweet vernal and ten were from browntop, which are new host records. Four isolates were obtained in survey three from lotus and subterraneum clover which are also new host records. Although it was observed to colonise seedlings of grasses and legumes it was non pathogenic to all tested hosts. This species was found to be common New Zealand pasture soils (Jackson 1965), and Thornton (1965) frequently isolated it from perennial ryegrass and less frequently from white clover. There are few other reports of this fungus being isolated from plant roots. *Gongronella butleri* are identified by the production of globose sporangia supported by a globose columella, and spores are oval but flattened on one side.

50. *Humicola fuscoatra* Traaen

Isolation site: Ruakura

Host: *Trifolium repens*

One isolate of *Humicola* was found on white clover roots at Ruakura. This genus is soil borne and widely distributed (Domsch *et al.* 1980), and has been reported from the rhizospheres of wheat, pineapple. *Humicola fuscoatra* is reported to be a root pathogen of tomato (De Gruyter *et al.* 1992) and has also been found to be a mycoparasite of vesicular-arbuscular mycorrhizal fungi (Daniels and Menge 1980). Jackson (1965) found this genus to be common in New Zealand pasture soils and another related species, *Humicola grisea* Traaen, has been isolated from the roots of perennial ryegrass (Thornton 1965), and Skipp *et al.* (1986) isolated it from red clover.

51. *Idriella bolleyi* (Sprague) Von Arx (= *Microdochium bolleyi* (Sprague) de Hoog and Heranides Nijhof)

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus; Lotus uliginosus ; Trifolium subterraneum.*

A total of 41 isolates of *Idriella bolleyi* were recorded from all four hosts sampled in survey one and two. It was more common at Ruakura with 18 isolates from ryegrass roots and seven isolates from white clover. At Whatawhata, nine isolates were recorded from sweet vernal, five from white clover and one each from ryegrass and browntop. It was also common in survey three with nine isolates being recorded from Yorkshire fog, lotus

and subterranean clover. *Idriella bolleyi* was found to be pathogenic to; red clover, white clover, subterranean clover, lotus, ryegrass, Yorkshire fog, cocksfoot, sweet vernal and browntop, and non pathogenic to soft brome, tall fescue and timothy. Extensive hyphal colonisation of cortex and epidermal root tissues was observed in most seedlings except those of timothy, which was not colonised. This fungus has been reported to strongly accumulate in rhizospheres of grass monocultures (Domsch *et al.* 1980), and has been isolated from the roots of perennial ryegrass and white clover (Skipp and Christensen 1989, Sarathchandra *et al.* 1995). *Idriella bolleyi* occurs frequently on roots of many graminaceous plants, and is associated with both healthy and diseased grass roots (Domsch and Gams 1972, Murray and Gadd 1981, Salt 1976, Sharp 1959, Sprague 1948) . In the USA it is reported to cause root necrosis of pasture grasses (including; browntop, cocksfoot, tall fescue, soft brome, timothy and Poa) and cereals (Farr *et al.* 1989). This fungus has also been investigated as a biocontrol agent against take all (*Gaeumannomyces graminis*) in wheat (Lascaris and Deacon 1991) and other cereals (Douglas and Deacon 1994), as it is a weak pathogen of cereals with the ability to control root pathogens by competitive exclusion from naturally senescing host tissues (Allan *et al.* 1992). It was also reported that *Idriella bolleyi* has a positive trophic response towards dying roots and a negative trophic response to living roots (Allan 1992).

52. *Idriella lunata* P.E. Nelson and Wilhelm

Isolation site: Whatawhata

Host: *Lolium perenne*

A single isolate *I. lunata* was obtained from perennial ryegrass which is a new host record. This species has also been isolated from low vigour pastures of Caucasian clover and white clover in the Bay of Plenty (Burch pers. comm.). This fungus is generally not widespread and there are few reports of it from plants roots (Domsch *et al.* 1980). However, it has previously been found to be pathogenic on strawberry roots (D' Ercole 1985) and was a component of the mycoflora of healthy spruce tree roots (Holdenrieder and Sieber 1992) . *Idriella lunata* is distinguished by the production of two-celled darkly pigmented chlamydospores on short denticles and filiform hyaline conidia similar to *Dactylaria acerosa.*

53. *Mariannaea elegans* (Corda) Samson (*Paecilomyces elegans* (Corda) Mason and Hughes

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Trifolium subterraneum; Bromus hordeaceus.*

Mariannaea elegans was relatively common on all hosts in surveys one and two, with 29 isolates being recorded, 10 from Ruakura and 19 from Whatawhata. Seven isolates were recorded from subterranean clover and soft brome in survey three. Roots of all tested hosts were colonised, with hyphae being observed in the epidermis tissue of all seedlings. No disease symptoms were observed on roots, so *M. elegans* is considered to be non pathogenic. Sporulation was stimulated in the presence of hosts seedlings as copious production of conidiophores was observed on the surface of infected epidermal cells. There are no previous reports of this fungus from New Zealand plants or pastures, and it has rarely been reported from roots in overseas studies.

54. *Metarhizium anisopliae* (Metschn.) Sorok.

Isolation site: Whatawhata

Host: *Anthoxanthum odoratum; Agrostis capillaris*

A widespread insect pathogen, *M. anisopliae* was obtained once from sweet vernal and once from browntop. In further *in vitro* tests hyphal colonisation of the epidermis of white clover, cocksfoot, sweet vernal and browntop roots was observed, and the fungus was non pathogenic to all tested hosts. It has been previously reported on Waikato pastures by di Menna and Parle (1970) who recorded it on the leaves of perennial ryegrass and white clover.

***Mortierella* Coemans**

Species of *Mortierella* are amongst the commonest recorded soil fungi (Domsch *et al.* 1980), and is frequently reported from pasture roots (Waid 1957, Gadgil 1965, Thornton 1965, Lubruyere 1979). Taxonomically they have been placed in the zygomycete family, Mortierellaceae, which produce sporangia with no columella and are split into spores. Six species of the genus were identified (entries 55-60 below), and was the most frequently isolated zygomycete genus recorded in this study. This genus is a competitive soil saprophyte and is frequently reported from numerous soil types. For example *M. alpina, M. elongata* and *M. hyalina* were common on soil particles in Dutch agricultural soils (Gams 1992). A total of 96 isolates were isolated in surveys one and two (Table 6), and a further six isolates were obtained in survey three.

Table 6. *Mortierella* species isolated from pasture plants in all three surveys.

Species	Ryegrass *	Clover *	Ryegrass	Clover	Browntop	Sweet Vernal	Total	%Total
M. alpina	2	3	3	-	-	-	**8**	**8.3**
M. baineri	1	1	-	-	-	-	**2**	**2.1**
M. elongata	4	4	-	-	4	2	**14**	**14.5**
M. gamsii	5	14	2	2	5	-	**28**	**29.2**
M. globulifera	7	5	13	9	3	5	**42**	**43.8**
M. hyalina	-	1	-	-	-	1	**2**	**2.1**
TOTAL	19	28	18	11	12	8	**96**	**100**

* Ryegrass and clover sampled from improved dairy pasture Ruakura, the remaining species sampled from unimproved hill pasture at Whatawhata

55. *Mortierella alpina* Peyronel

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Bromus hordeaceus*

Eight isolates of *M. alpina* were recorded from clover and ryegrass, with additional three isolates identified from soft brome in survey three. It is reported to be the commonest *Mortierella* species isolated from soil (Domsch *et al.* 1980), and Thornton (1965) isolated it from perennial ryegrass and white clover. There are also other reports of this fungus from the root regions of grasses (Lim 1969), potato, juniper and wheat (Domsch *et al.* 1980). (Hay and Skipp 1993) recorded this fungus on nematode cysts from North Island pasture soils. In pathogenicity tests *M. alpina* was non pathogenic to all pasture seedlings except soft brome where epidermal tissues were colonised with conspicuous discoloration and lesioning being observed on infected roots.

56. *Mortierella bainieri* Cost

Isolation site: Ruakura

Host: *Trifolium repens, Lolium perenne.*

Two isolates of *M. bainieri* were recorded at Ruakura in survey one, and these are new host records for both ryegrass and clover. Previous isolation records of this fungus show it is common on decaying agarics, where it causes shaggy stipe disease (Fletcher 1973), but is also found in agricultural soils, waterlogged pastures, grasslands and heathland (Domsch *et al.* 1980).

57. *Mortierella elongata* Linnem

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris;*

Trifolium subterraneum.

Mortierella elongata was found on all four hosts, at both Whatawhata and Ruakura plots and on subterranean clover in survey three. However, it was still uncommon, with only 14 isolates being recorded. *In vitro* tests found this fungus to invade the epidermis of; white clover, soft brome, lotus, tall fescue, sweet vernal, Yorkshire fog and red clover, but was non pathogenic to all tested hosts. In rhizosphere and root studies it has been reported from perennial ryegrass and white clover roots (Thornton 1965), potato, wheat, poplar and other crops (Domsch *et al.* 1980).

58. *Mortierella gamsii* Milko.

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Agrostis capillaris; Bromus hordeaceus.*

The second most common species of the genus was *M. gamsii* with 28 isolates recorded from white clover (seven isolates), ryegrass (16 isolates), browntop (five isolates). Two isolates were recorded from soft brome in survey three. The fungus was observed to invade epidermal tissues of pasture seedlings and *in vitro* tests also showed this species to be pathogenic to; subterranean clover, white clover, soft brome, lotus and red clover, mildly pathogenic to; Yorkshire fog, ryegrass, sweet vernal, timothy and browntop, and non pathogenic to tall fescue and cocksfoot. *Mortierella gamsii* is one of the most widely distributed *Mortierella* species and has been reported from tussock-grassland, forest and grassland soils in New Zealand (Thornton 1958, 1960) and is also common in forest and grassland soils worldwide (Domsch *et al.* 1980). It has been recorded from ryegrass roots (Thornton 1965) and from the rhizospheres of wheat, and grasses (Domsch *et al.* 1980).

59. *Mortierella globulifera* Rostrup

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris*

Mortierella globulifera was the commonest species of this genus isolated in this study, with 42 isolates being obtained from white clover, ryegrass, sweet vernal and browntop. Epidermal invasion was observed in the roots of; ryegrass, white clover, subterranean clover, lotus, tall fescue, sweet vernal, Yorkshire fog, cocksfoot and red clover. Some mild disease symptoms were seen on ryegrass and subterranean clover, but *M. globulifera* was non pathogenic to all remaining hosts. This is fungus has rarely been reported from roots so all are new hosts records.

60. *Mortierella hyalina* (Harz.) W. Gams

Isolation site: Whatawhata; Ruakura

Host: *Lolium perenne; Anthoxanthum odoratum.*

Mortierella hyalina was infrequently found in this study as only two isolates were recorded, one at Ruakura on ryegrass and the other at Whatawhata on sweet vernal which is a new host record. Thornton (1965) also reported this species from the roots of ryegrass.

61. *Mucor* Mich ex St.

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus; Trifolium subterraneum.*

A total of 43 isolates of *Mucor* were identified from all three surveys. Species of *Mucor* are widely reported from soils and rhizopheres of plants worldwide (Domsch *et al.* 1980). *Mucor heimalis* Wehmer has previously been reported from pasture ryegrass roots (Humphreys-Jones and Waid 1963, Thornton 1965, Sarathchandra *et al.* 1995). Gadgil (1965) also reported *Mucor* from the roots of ryegrass, cocksfoot and meadowgrass. This genus, along with *Gongronella* and *Mortierella,* were the only genera of Zygomycetes isolated in the surveys, and together this group comprised 3% of the total fungi recorded. Zygomycetes are one of the commonest groups of soilborne fungi and this study also confirms them as important root colonising fungi of pasture roots. Other Zygomycete genera reported from pasture roots include; *Absidia, Cunninghamella, Zygorrhynchus, Actinomucor, Rhizomucor* and *Rhizopus* (Waid 1957, Gadgil 1965, Thornton 1965, Labruyere 1979, Domsch *et al.* 1980). Zygomycetes are generally regarded to colonise root surface and outer root cortex of plant hosts (Waid 1957, Skipp and Christensen 1989).

62. *Myrothecium verrucaria* (Alb and Schw. : Fr) Ditmar

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne*

Myrothecium verrucaria was relatively uncommon as only 14 isolates were found on the roots of ryegrass (eight isolates) and white clover (six isolates), however it was present at both pasture sites. In pathogenicity tests, *M. verrucaria* re-invaded seedling roots of pasture seedlings and was observed to be pathogenic to; ryegrass, subterranean clover, white clover, red clover, timothy, Yorkshire fog and cocksfoot, mildly pathogenic to; lotus, tall fescue, sweet vernal and browntop, and non pathogenic to soft brome. *Myrothecium verrucaria* has rarely been reported from pasture roots, although it has been isolated from subterranean clover foliage in Australia (Barbetti 1984b) and was shown to cause root rot under non competitive conditions. It has also been found on bean roots (Waid 1974) , the

rhizospheres of wheat, oat, barley, lucerne and other crops (Domsch *et al.* 1980) and clover leaves in New Zealand pastures (di Menna and Parle 1970). It has been reported to have a large host range, infecting 54 plant species in 42 genera after artificial inoculation (Yang and Jong 1995) and was investigated as a potential mycoherbicide of several weeds. The same study also reported *M. verrucaria* to be a facultative parasite of pasture species such as red clover, lucerne, lotus, canary grass and several *Poa* species.

***Paecilomyces* Bain.**

Three species of *Paecilomyces* were identified from pasture roots (entries 63-65), and 113 isolates (1.5%) were recorded from all three surveys. The genus is closely related to *Penicillium,* but is distinguished by its divergent phialides which possess a swollen base. There are a few reports of this genus from pasture roots and soils in New Zealand (Jackson 1965, Bonish unpublished), but this study clearly showed *Paecilomyces* to be an important component of the pasture root mycoflora.

63. *Paecilomyces carneus* Duche and Heim

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus; Bromus hordeaceus.*

Paecilomyces carneus was isolated 37 times from white clover (14 isolates), ryegrass (nine isolates), sweet vernal (five isolates), browntop (nine isolates), and it also occurred on Yorkshire fog (two isolates) and soft brome (one isolate) roots in survey three. It was found to be non pathogenic to all tested grass and legume seedlings and was observed to colonise the epidermis of; ryegrass, subterranean clover, white clover, red clover, Yorkshire fog, cocksfoot, timothy, sweet vernal, tall fescue and browntop, all of which are new hosts records in New Zealand. It has been isolated from the rhizosphere of grasses and cereals (Domsch *et al.* 1980), and although regarded as a frequent soil fungus, there are comparatively few published reports of its occurrence. Gams (1992) reported it to be frequently isolated from Dutch agricultural soils.

64. *Paecilomyces lilacinus* (Thom) Samson

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus; Lotus uliginosus* .

Paecilomyces lilacinus was the most frequently isolated species of this genus with 62 isolates being recorded from all surveys. *In vitro* pathogenicity and root colonisation tests of pasture seedlings, showed this fungus could invade epidermal tissues of grasses and legumes and was assessed as mildly pathogenic to seedlings of; ryegrass, subterranean

clover, white clover, soft brome, lotus, timothy, and browntop. It has previously been reported from the roots of wheat, barley, sugarcane and banana (Domsch *et al.* 1980). *Paecilomyces lilacinus* has been rarely reported from plant roots in New Zealand, but it was common on the cysts of the nematode *Heterodera trifolii* in North Island pasture soils (Hay and Skipp 1993).

65. *Paecilomyces marquandii* (Massee) Hughes
Isolation site: Whatawhata; Ruakura
Host: *Trifolium repens; Lolium perenne.*

This species was recorded 11 times in surveys one and two. Ten isolates were found on ryegrass at both sites, and one isolate was recorded from white clover at Whatawhata. *Paecilomyces marquandii* was non pathogenic to tested hosts and colonised the epidermal root tissues of all pasture grasses and legumes. Jackson (1965) reported the occurrence of this species from pastoral soils in New Zealand but there are no previous reports of its presence in roots.

Penicillium Link ex Fr.

Species of *Penicillium* are ubiquitous saprophytes whose conidia are easily distributed through the atmosphere and also predominate in soils of temperate regions (Domsch *et al.* 1980). The genus is frequently reported from all soil types, ranging from agricultural soils (Gams 1992) to forest soils (Soderstrom and Baath, 1978, Widdens 1986). However this genus has been reported to be more frequently isolated from soils with a low moisture content (Dix and Webster 1995). *Penicillium* was the most diverse genus isolated in this study, with 19 species being identified and a further six isolates unidentified to species level. One of unidentified species isolated from ryegrass (Appendix 2) resembled *P. wacksmannii* Zaleski, as it produced divergent biverticillate conidiophores as well as monoverticillate conidiophores, but most of their colony diameters and growth at 5°C on CYA, were different to Pitt's key (1979). The other five unidentified isolates were isolated from clover at Whatawhata, and were similar to *P. corylophilum* Dierckx, but further taxonomic examination is needed to differentiate these five isolates from the morphologically similar *P. citrinum.*

A total of 198 *Penicillium* isolates were recorded from white clover, ryegrass, sweet vernal and browntop in surveys one and two (table 7), 3% of all fungi isolated, and in survey three, 21 isolates were recorded (table 8). Although a large number of penicillia were recorded, only three species, *P. chrysogenum, P. janthinellum* and *P. simplicissimum,* were isolated in significant numbers from all hosts. *Penicillium brevicompactum* was also isolated from most hosts and sites but in very low numbers, and the remaining 15 species were uncommon and were often only recorded once. Previous studies on pasture root

mycoflora have reported the frequent isolation of penicillia from roots and the rhizosphere (Waid 1957, Gadgil 1965, Thornton 1965, Falloon 1985), and other studies report less frequent isolation from pasture roots (Skipp and Christensen 1989, Sarathchandra *et al.* 1995). Although species of penicillia and its related genera, *Aspergillus* and *Paecilomyces,* are considered to be saprophytes, several penicillia have been reported to be pathogenic to agricultural crops such as wheat (Dewan and Sivasithamparam 1988a, Harman and Pfleger 1974), maize (Caldwell *et al.* 1981), subterranean clover (Barbetti 1984a) red clover and lucerne (Mackinaite and Strukcinskas 1992). Many penicillia have also been reported to be antagonistic to other root fungi including important root pathogens (Dodd and Stewart 1992, Fehrmann *et al.* 1977, Johansson and Marklund 1980, Kommedahl and Windels 1978, Kovacikova and Kudela 1990).

Table 7. *Penicillium* species isolated from roots in surveys one and two.

Species	Ryegrass *	Clover *	Ryegrass	Clover	Browntop	Sweet Vernal	**Total**	**%Total**
P. atramentosum	2	-	-	4	-	-	**6**	**3.0**
P. brevicompactum	-	1	4	1	1	2	**9**	**4.5**
P. chrysogenum	8	3	2	3	13	1	**30**	**15.0**
P. citrinum	1	-	-	-	2	-	**3**	**1.5**
P. crustosum	1	1	-	-	-	-	**2**	**1.0**
P. decumbens	2	-	-	-	1	-	**3**	**1.5**
P. glabrum	-	-	1	-	-	-	**1**	**0.5**
P. griseofulvum	1	-	-	-	-	-	**1**	**0.5**
P. islandicum	1	-	-	-	-	1	**2**	**1.0**
P. janczewskii	-	-	1	-	-	-	**1**	**1.0**
P. janthinellum	6	1	26	4	35	4	**76**	**38.3**
P. minioluteum	-	-	-	-	1	-	**1**	**0.5**
P. simplicissimum	4	3	15	6	15	13	**56**	**28.3**
P. variabile	-	-	-	-	1	-	**1**	**0.5**
Penicillium sp.	-	-	-	5	1	-	**6**	**3.0**
TOTAL	26	9	48	23	70	21	**198**	**100**

Results from survey three again show *P. chrysogenum, P. janthinellum* and *P. simplicissimum* to be the most frequently isolated penicillia (table 8), as well as *P. raistrickii* which was not obtained in either survey one or two.

Table 8. *Penicillium* species isolated from roots in survey three

SPECIES	Subterranean clover	Lotus	Yorkshire fog	Soft brome	Total	Total %
P. brevicompactum	-	1	-	-	**1**	**4.8**
P. chrysogenum	-	-	-	5	**5**	**23.8**
P. dendriticum	-	-	-	1	**1**	**4.8**
P. expansum	1	-	-	-	**1**	**4.8**
P. janthinellum	2	-	2	-	**4**	**19.0**
P. oxalicum	-	1	-	-	**1**	**4.8**
P. rastrickii	1	-	-	2	**3**	**14.2**
P. rugulosum	1	-	-	-	**1**	**4.8**
P. simplicissimum	1	-	-	3	**4**	**19.0**
TOTAL	6	2	2	11	**21**	**100**

In vitro tests showed that the roots of most pasture hosts were recolonised by inoculated penicillia, but hyphal penetration of roots was limited to the epidermis of most hosts (table 9). Exceptions were *P. variabile* which was observed in the cortex of subterranean clover, and *P. janthinellum* and *P. simplicissimum,* which colonised the cortex of tall fescue.

Table 9. Root colonisation of pasture seedlings by *Penicillium* species.

Species	WC*	SC	RC	LO	RG	TF	GG	TI	SV	BT	CF	YF
P. brevicompactum	1	1	1	1	1	1	0	1	1	1	1	1
P. chrysogenum	1	1	1	1	1	1	1	1	1	1	1	1
P. decumbens	1	1	1	1	0	1	0	1	1	0	1	1
P. griseofulvum	1	1	0	0	0	1	0	1	1	1	1	1
P. janczewskii	1	1	1	1	1	1	1	1	1	1	1	1
P. janthinellum	1	1	1	1	1	2	1	1	1	1	1	1
P. oxalicum	1	1	1	1	1	0	0	1	1	0	1	1
P. simplicissimum	1	1	1	1	1	2	1	1	1	1	1	1
P. variabile	1	2	1	1	0	1	0	1	1	1	1	1

* WC white clover, SC subterranean clover, RC red clover, LO lotus, RG ryegrass, TF tall fescue, GG soft brome, TI timothy, SV sweet vernal, BT browntop, CF cocksfoot, YF Yorkshire fog.

66. *Penicillium atramentosum* Thom

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne*

The isolation of *P. atramentosum* has rarely been reported, and so is considered a rare species (Pitt, 1979), it has previously unreported from plant roots.

67. *Penicillium brevicompactum* Dierckx

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Lotus uliginosus .*

Penicillium brevicompactum is a cosmopolitan species, especially in soil, but never particularly frequent (Domsch *et al.* 1980). This was a characteristic observed in these surveys as it was present at both pasture sites on five hosts, but in very low numbers. *Penicillium brevicompactum* was observed to be mildly pathogenic to subterranean clover and lotus seedlings, but was non pathogenic to the remaining hosts tested. Dewan and Sivasithamparam (1988a) isolated this fungus from wheat and ryegrass roots, and found that it was pathogenic to ryegrass, decreasing shoot and root weights. It was also reported that wheat roots inoculated with *P. brevicompactum* were shorter and deformed compared to control plants.

68. *Penicillium chrysogenum* Thom

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Bromus hordeaceus.*

This species was the third most frequently isolated *Penicillium* and is a widely distributed soil fungus. It was observed in the epidermis of all seedlings (table 9), but was non pathogenic to all grass and legume hosts tested, a similar result reported by Falloon (1985), who isolated non pathogenic strains of *P. chrysogenum* from perennial and Italian ryegrasses. It has been isolated from wheat and ryegrass roots in Australia (Dewan and Sivasithsamparam 1988a), and was again found to be non pathogenic to plants. There are many isolation reports of this fungus from the rhizospheres of plants such as clover, lucerne, wheat, flax, trees and vegetable crops (Domsch *et al.* 1980).

69. *Penicillium citrinum* Thom

Isolation site: Whatawhata, Ruakura

Host: *Lolium perenne; Agrostis capillaris.*

Three isolates were recorded from survey one (table 6), and *P. citrinum* has been unreported from roots of pasture plants until now, although it has been isolated from the roots of sugar beet (Domsch *et al.* 1980). This species has previously been isolated from the common Waikato soil types (di Menna pers. comm.), including the two soils, Horotiu sandy loam and Te kowhai silt loam, which were sampled at Ruakura sites as part of this study. Pitt (1988) suggested this species was one of the most common eukaryotic life forms on earth, as it is ubiquitous in air, soil, decaying vegetation. It is also a powerful biodeteriogen, as it has been commonly reported to cause decay and losses to foods, textiles, plastics and paints (Kozakiewicz 1992).

70. *Penicillium crustosum* Thom

Isolation site: Ruakura

Host: *Trifolium repens; Lolium perenne*

Penicillium crustosum is regarded primarily as a weak pathogen of pomaceous fruits and a ubiquitous spoilage organism (Pitt 1979), it is therefore rarely recorded on plant roots. Only two isolates were identified here, both from ryegrass roots at Ruakura.

71. *Penicillium decumbens* Thom

Isolation site: Whatawhata, Ruakura

Host: *Lolium perenne; Agrostis capillaris.*

Penicillium decumbens invaded epidermal tissues of pasture seedling roots (except ryegrass, soft brome and browntop), and was non pathogenic to all hosts. Domsch *et al.* (1980) report this species has been isolated from the roots of *Pinus,* and the rhizospheres of clover, and groundnuts. It is unreported from plant roots in New Zealand and so its isolation from ryegrass and browntop are new host records. This is reported to be a ubiquitous soil fungus which is also common in decaying vegetation and foods, being previously reported from diverse geographic areas such as USSR, Egypt and Australia (Pitt 1988). *Penicillium decumbens* was distinctive in that it was one of the few species of monverticillate penicillia isolated from roots.

72. *Penicillium dendriticum* Pitt

Isolation site: Whatawhata

Host: *Bromus hordeaceus*

One isolate of *P. dendriticum* was obtained from soft brome, which is a new host record. This fungus is also unreported from plant roots in New Zealand.

73. *Penicillium expansum* Link ex Gray

Isolation site: Whatawhata

Host: *Trifolium subterranean*

Most previous records of this penicillia in New Zealand are from rotting citrus fruit (Pennycook 1989), and it is also reported to be the most frequent *Penicillium* species on fruit as well as being a widely distributed soil fungus (Domsch *et al.* 1980). The identification of this single isolate from subterranean clover is another new host record.

74. *Penicillium glabrum* (Wehmer) Westling

Isolation site: Whatawhata

Host: *Lolium perenne*

Pitt (1979) describes this *Penicillium* as "the most common penicillia on earth", being isolated from numerous substrates, however a single isolate was recorded from ryegrass and there few reports of it from roots. This species is considered cosmopolitan and is widely distributed in soils (Pitt 1988), although a closely related and morphologically similar species, *P. spinulosum* Thom, is considered by Pitt to be much more common. *Penicillium glabrum* produces monverticillate conidiophores, which are the simplest penicillus type as there is only one branch point in the conidiophore, and all monverticillate species are classed in the sub genus *Aspergilloides.*

75. *Penicillium griseofulvum* Dierckx

Isolation site: Ruakura

Host: *Lolium perenne*

Penicillium griseofulvum was isolated from wheat and ryegrass roots in Australia (Dewan and Sivasithamparam 1988a), and it caused the death of these hosts seedlings. It has a world wide distribution and plays a major role in the decay of vegetation, seeds, cereals and foodstuffs (Kozakiewicz 1995). In this study a single isolate was recorded from ryegrass and it was observed to invade epidermal root tissues but was non pathogenic to all tested hosts.

76. *Penicillium islandicum* Sopp

Isolation site: Whatawhata

Host: *Lolium perenne; Anthoxanthum odoratum*

Two isolates of *P. islandicum* were obtained, one each from ryegrass and sweet vernal. The isolation of this penicillia from ryegrass and sweet vernal are new host records. It has a worldwide distribution, and has often been isolated from agricultural and cultivated soils (Kozakiewicz 1992), in rhizosphere studies it has been associated with lucerne, maize, rice and groundnut (Domsch *et al.* 1980). Pitt (1979) declares this fungus to be more commonly reported from tropical areas than from temperate zones, but it has been previously isolated from Waikato soils (di Menna pers. comm.). It is reported to be xerophilic and is therefore a active agent in grain and cereal spoilage (Pitt 1988).

77. *Penicillium janczewskii* Zaleski

Isolation site: Whatawhata

Host: *Lolium perenne*

This species invaded the epidermis of all tested hosts and was observed to be mildly pathogenic to sweet vernal, causing conspicuous browning of roots with some lesioning but was non pathogenic to all remaining hosts. It has been frequently isolated from New Zealand soils (di Menna pers. comm.), with soil being reported as its major and almost exclusive habitat (Pitt 1979). It was also reported to be the most common *Penicillium* isolated from Dutch agricultural soils (Gams 1992).

78. *Penicillium janthinellum* Biourge

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus; Trifolium subterraneum; Bromus hordeaceus.*

Penicillium janthinellum was the most frequently recorded penicillia in both surveys, as 76 isolates were obtained in survey one and six from survey two. *In vitro* pathogenicity tests showed it to be pathogenic to red clover causing root rot and lesions, and was mildly pathogenic to lotus and Yorkshire fog. It has previously been found to be pathogenic on Italian and perennial ryegrass (Falloon 1985), and on wheat and ryegrass *Lolium rigidum* Gaud. (Dewan and Sivasithamparam 1988a), where it was shown to rot the roots of these plants. Thornton (1965) isolated it from the roots of perennial ryegrass. In New Zealand soils, it was found to be the most commonly occurring *Penicillium* (di Menna pers. comm.). *Penicillium janthinellum* has been reported to be antagonistic to other root pathogens such as *Fusarium oxysporum* (Fehrmann *et al.* 1977). *Penicillium janthinellum* produces irregular branching biverticillate conidiophores and is therefore classed in the sub genus *Furcatum* section *Divaricatum,* and because of its variable branching and frequent production of monverticillate conidiophores, the species can initially be difficult to identify. Pitt (1988) reports this species to be the most morphologically variable penicillia.

79. *Penicillium minioluteum* Dierckx

Isolation site: Whatawhata

Host: *Agrostis capillaris*

Falloon (1985) isolated a single isolate of *Penicillium minioluteum* from perennial ryegrass and found it to be pathogenic to this host as well as Italian ryegrass. Apart from this record it has not been previously isolated from plants in New Zealand. The isolation of this single isolate from browntop is therefore another new host record. *Penicillium minioluteum* is primarily a soil fungus and can cause rapid biodeterioration in damp situations (Pitt 1988).

80. *Penicillium oxalicum* Currie and Thom

Isolation site: Whatawhata

Host: *Lotus uliginosus*

A single isolate was obtained from lotus, which is another new host record. It has been previously isolated from the roots of cotton and wheat, and from the rhizospheres of many other plant hosts (Domsch *et al.* 1980), but there are no reports of this fungus from roots or pasture in New Zealand. This species was reported to be as effective as fungicide for controlling root rot of peas as it was antagonistic to *Aphanomyces eutiches* Drechsler, *R. solani* and several fusaria (Kaiser and Hannan 1984, Kommedahl and Windels 1978). This species is also reported to be a toxicogenic pathogen as it produces a toxin (oxalic acid) which causes root disease symptoms without primary infection of root tissues (Kommedahl and Windels 1979).

81. *Penicillium raistrickii* G. Smith

Isolation site: Whatawhata

Host: *Trifolium subterranean*

Penicillium raistrickii has been reported from a variety of substrates, but the isolation from subterranean clover roots is previously unreported. Patterson *et al.* (1981) reported this species as one of the soil fungi from pastures that are associated with grass stagger symptoms, as it produces tremorgenic toxins. It has also been reported to suppress damping-off root disease of beet root (Dodd and Stewart 1992).

82. *Penicillium rugulosum* Thom

Isolation site: Whatawhata

Host: *Trifolium subterranean*

Penicillium rugulosum has not previously been reported from pasture roots, but has been isolated from the rhizospheres of lucerne, cereals, rice and peas (Domsch *et al.* 1980), and has been isolated from plant roots and soil in Polish grasslands (Chrusciak *et al.* 1977).

83. *Penicillium simplicissimum* (Oudem.) Thom

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Trifolium subterraneum; Bromus hordeaceus.*

With a total of 60 isolates being recorded in this study, *P. simplicissimum* was the second most frequently isolated *Penicillium* species. This species is a broadly circumscribed taxon (Pitt 1988), and can be extremely variable in culture. Some of these root-colonising isolates grew at 37°C on CYA while others did not, and Pitt (1988) has reported that many isolates from Australasia do not grow at this temperature, whereas Northern hemisphere isolates do grow at 37°C. These root isolates also grew at 5°C, which is another difference between Australasian and Northern hemisphere strains. *Penicillium simplicissimum* was found to be mildly pathogenic to ryegrass and soft brome, producing marked discoloration to inoculated seedlings. Falloon (1985) also reported *P. simplicissimum* to be pathogenic to perennial ryegrass as well as Italian ryegrass. In addition to pasture roots, it has been recorded from Te Kowhai silt loam soil in the Waikato (di Menna pers. comm.). *Penicillium simplicissimum* was assessed to be antagonistic to *Pythium* (which causes damping-off of seedlings), and therefore it has also been investigated as biocontrol agent (Dodd and Stewart 1992).

84. *Penicillium variabile* Sopp

Isolation site: Whatawhata

Host: *Agrostis capillaris*

The single isolate found on browntop roots was able to colonise the root epidermis of most hosts except ryegrass and soft brome, and was mildly pathogenic to subterranean clover causing root surface lesions. *Penicillium variabile* is a ubiquitous species, and is the most commonly encountered member of the sub genus *Biverticillium* (Pitt 1988). It is well documented from soil (Pitt 1979), and has also been reported to be antagonistic to clover root pathogens such as *Phytophthora cinnamomi* Rands. and fusaria (Kovacikova and Kudela 1990).

85. *Periconia macrospinosa* Lefebvre and A. G. Johnson

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus; Lotus uliginosus ; Trifolium subterraneum; Bromus hordeaceus.*

Periconia macrospinosa was frequently isolated from pasture roots and was common on all hosts sampled. A total of 124 isolates were identified from all surveys and it was the most frequent fungus isolated from Yorkshire fog comprising 20% of all isolates from

that pasture plant. Many isolates only sporulated after being subjected to near-UV light on hay agar for seven days. Seedling roots of; subterranean clover, timothy, browntop and red clover, were systemically penetrated, as hyphae of *P. macrospinosa* were observed in vascular tissues. All other host roots were also invaded, but hyphae were only observed in the epidermis or outer cortex. The production of chlamydospores inside root tissue was frequently observed. *Periconia macrospinosa* was pathogenic to red clover and mildly pathogenic to ryegrass, browntop and subterranean clover. There are mixed reports on its pathogenicity, for example Falloon (1985) and Skipp and Christensen (1989), report it to be non pathogenic to ryegrass, while (Glynne 1939) and (Scott *et al.* 1979) reported it to be pathogenic to wheat. *Periconia macrospinosa* is by far the most commonly soil borne species of its genus in the temperate zone (Domsch *et al.* 1980), and has been most frequently reported from the rhizosphere and roots of various plants, including grasses (Opperman and Wehner 1994), cereals (Hall 1986, Kirk and Deacon 1987a, Sturz and Bernier 1987) , sorghum (Odvody and Dunkle 1984) and maize (Falloon 1982, Fowler 1985). Other surveys of ryegrass and clover pasture roots in New Zealand all report the frequent occurrence of *P. macrospinosa* on roots (Thornton 1965, Skipp and Christensen 1982, Skipp and Christensen 1989). Another species, *P. circinata* (Mangin) Sacc. has also been isolated from roots of pasture species such as columbus grass (Dunkle 1992) .

86. *Pestalotia* De Not.
Isolation site: Ruakura
Host: *Lolium perenne*

A single isolate of *Pestalotia* was recorded on ryegrass roots. This species is a Coelomycete producing carbonaceous and simple acervuli, which bear darkly pigmented, 5- septate conidia of which the apical cell is hyaline and setulate. Isolates of this fungus have been reported in Waikato pastures on the leaves of white clover and perennial ryegrass (di Menna and Parle 1970). It has previously been reported as a root rot pathogen of many plants such as; fruit tree seedlings (Dzagnidze and Purtseladze 1979) , iceplant (MacDonald *et al.* 1984) and red clover (Nagovitsyna 1977).

87. *Phymatotrichum omnivorum* (Shear) Duggar (= *Phymatotrichopsis omnivorum* (Duggar) Hennebert)
Isolation site: Ruakura
Host: *Lolium perenne*

The genus *Phymatotrichum* was created in 1885, being derived from the greek words *phymato* (tumor-like) and *tricho* (hair). It is a soilborne Deuteromycete placed in the order Moniliales and produces blastoconidia on sterigmata on spheroidal conidiophores which in turn arise from thick walled hyphae. The fungus is reported to rarely sporulate *in vitro* (Lyda and Kenerley 1992) however in this study, masses of buff coloured conidia and dark

green to black microsclerotia were produced on PCA. Sporulation was also induced using Bonner and Addicotts medium (BAM, appendix 1), a medium specifically used for this purpose (Woods *et al.* 1967). A single isolate of *P. omnivorum* was found on ryegrass roots in survey two. From *in vitro* tests, it was found that all grass and clover seedlings were colonised by *P. omnivorum* and inoculation of the fungus onto these water agar seedling plates was another good way to trigger *in vitro* sporulation. It was observed to be pathogenic to; white clover, red clover, ryegrass, Yorkshire fog, timothy, cocksfoot, sweet vernal and browntop, mildly pathogenic to; subterranean clover, lotus and tall fescue but non pathogenic to soft brome. *Phymatotrichum omnivorum* probably has the broadest host range of any known soilborne fungus (Lyda and Kenerley 1992), attacking thousands of plant species (Blank 1953; Domsch *et al.* 1980) . It is reported to be less pathogenic to monocotyledonous plants but is still capable of parasitizing the tissue (Lyda and Kenerley 1992). Overseas it has been found on; cotton, Bermuda grass (*Cynodon dactylon* L.), Johnsongrass (*Sorghum halepense* L.) (Domsch *et al* 1980, Lyda and Kenerley 1992) and the causal agent of root rot on lotus, red clover and white clover (Farr *et al.* 1989). It has rarely been reported outside North America and to date has been unreported from New Zealand.

88. *Pithomyces chartarum* (Berk. and Curt.) M. B. Ellis

Isolation site: Whatawhata

Host: *Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris*

This dematiaceous hyphomycete was isolated three times in survey one from the roots of ryegrass, sweet vernal and browntop.. It is a cosmopolitan fungus being isolated from soil, air and is especially frequent on fodder grasses in pastures, where it has been found to cause facial eczema of sheep (Ellis 1971) . It has been mainly reported from dead plant leaves of over 50 plant species (Ellis 1971), however *in vitro* tests showed it could colonise the epidermis of; ryegrass, white clover, tall fescue, sweet vernal and Yorkshire fog but was non pathogenic to these and other hosts tested.

89. *Plectosporium tabacinum* van Beyma (teleomorph =*Plectosphaerella cucumerina* (Lindfors) W. Gams).

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris*

Plectosporium tabacinum was isolated 17 times in this study and it was found on ryegrass, sweet vernal and browntop from Whatawhata, and from clover and ryegrass from Ruakura. Sexual structures of the teleomorph, *Plectosphaerella cucumerina,* were not produced in culture but it was able identified using the morphology of asexual structures. Until recently, the systematics of the anamorph had been problematic. The anamorph was initially named as *Cephalosporium tabacinum* (van Beyma), but was subsequently named

as *Fusarium tabacinum* (van Beyma) W. Gams, and *Microdochium tabacinum* by von Arx (Gams 1993). This species could not be correctly accommodated in any of these genera, which lead to its current reclassification into a new genus, *Plectosporium* (Palm *et al.* 1995). *Plectosporium tabacinum* produce one-septate conidia which were curved at each end, from single phialides. Colonies were aplanate and pinkish in colour, and conidia were copiously produced on PCA, which gave colonies a moist slimy appearance. From *in vitro* tests it was observed to be pathogenic to; red clover, white clover, subterranean clover, ryegrass, Yorkshire fog, lotus and sweet vernal, mildly pathogenic to; cocksfoot and browntop, and non pathogenic to; timothy, soft brome and tall fescue. *Plectosporium tabacinum* is a very common fungus found in soil and decaying plant material, and has also been reported from the rhizospheres of wheat, grasses, flax and sugar beet (Domsch *et al.* 1980). It has been implicated to cause root and wilt diseases in a number of plant hosts such as sunflower (*Helianthus annuus* L.), cucumber (*Cucumis sativus* L.), basil (*Ocimum basilicum* L.), tomato (*Lycopersicon esculentum* Miller) and peanuts (*Arachis hypgaea* L.) (Palm *et al.* 1995). This fungus can exist as a soil saprophyte and was the second most common species isolated from Dutch agricultural soils (Gams 1992), where it has also been isolated from ryegrass roots in old pastures (Labruyere 1979). There are no previous reports of it from roots in New Zealand pastures.

90. *Preussia aemulans* (Rehm) von Arx

Isolation site: Whatawhata; Ruakura

Host: *Lolium perenne; Anthoxanthum odoratum*

Preussia Fuckel is an ascomycete genus placed in the Sporormiaceae family which produce mostly non ostiolate ascomata with four celled ascospores. *Preussia aemulans* produced flask shaped ascomata with cylindrical asci containing eight ascospores in each ascus. Although reports of this species are rare, with even fewer reports of it on roots, 24 isolates were obtained in this study. It was mostly isolated from ryegrass at Ruakura (survey two) where 18 isolates were recorded, a further six isolates were obtained at Whatawhata on sweet vernal which is a new host record. The fungus colonised the epidermis and outer cortex of seedling roots in pathogenicity tests and ascomata were copiously produced at the agar/ root interface. *Preussia aemulans* was non pathogenic to all inoculated pasture hosts, apart from red clover, which was systemically penetrated by hyphae and caused root rot damage to all seedlings.

91. *Pseudallescheria boydii* (Shear) McGinnis *et al.* (= *Petriellidium boydii* (Shear) Malloch , = *Monosporium apiosporum* Sacc., = *Scedosporium apiospermum* (Sacc.) Sacc. ex Castell and Chalmers).

Isolation site: Whatawhata; Ruakura

Host: *Lolium perenne; Anthoxanthum odoratum*

Pseudallescheria boydii belongs to the Ascomycete family, Microscaceae Lutteral ex Malloch, which is closely related to the Chaetomiaceae. Its anamorph is known as *Scedosporium apiospermum*, and produces solitary conidia on single conidiophores. A total of 12 isolates of the anamorph *S. apiospermum* were identified, 11 from ryegrass at Ruakura and one from sweet vernal roots at Whatawhata. The teleomorph stage was also produced in culture. The fungus is soilborne (Domsch *et al.* 1980), and is reported to have a world wide distribution often being associated with deep seated mycoses in animals.

92. Pycnidial fungi

Pycnidial fungi were frequently encountered from roots in this study being present on most species sampled. Pycnidial fungi are classed as Coelomycetes within the Deuteromycetes and produce asexual conidia (usually aseptate) within pycnidia. A total of 135 isolates (2%) of pycnidial fungi were isolated from the surveys and so were ubiquitous on pasture roots. *Phoma medicaginis* Malbranche and Roumeguere comprised over 50% of isolates but more morphological study was needed to confirm this identification along with the other unidentified species of *Phoma* and *Phomopsis* that were present, but was beyond the scope of this investigation. Other mycological studies on the roots of pasture plants in New Zealand frequently record the presence of pycnidial fungi, particularly *Phoma* species (Thornton 1965, Skipp and Christensen 1982, 1983, 1989, Falloon 1985, Sarathchandra *et al.* 1995). Some species such as *Phoma exigua* Desm. and *Phoma chrysanthemicola* Hollos have been reported to be pathogenic to white clover (Skipp and Christensen 1982), red clover (Skipp *et al.* 1986) and mildly pathogenic to ryegrass (Skipp and Christensen 1989) which show future research on the identification and pathogenicity assessments of these isolates obtained from Waikato pastures would be valuable.

93. *Pyrenophora* Fr.

Isolation site: Ruakura

Host: *Lolium perenne*

Three isolates of *Pyrenophora* were recorded from ryegrass roots at Ruakura. This ascomycete genus is the teleomorph of *Dreschlera* (see entry 34). Production of ascomata readily occurred on HA. Most reports of this genus from New Zealand pastures have been as leaf colonisers (McKenzie 1978, Pennycook 1989), but there are no previous reports of it from roots.

94. *Pyricularia oryzae* Cav.

Isolation site: Whatawhata

Host: *Trifolium repens; Anthoxanthum odoratum; Agrostis capillaris*

Pyricularia oryzae is a dematiaceous hyphomycete which was isolated six times from three hosts at Whatawhata, four of the isolates occurring on sweet vernal which is a new host record. This species is characterised by the production of solitary, pyriform, two-septate conidia from single conidiophores. It is reported on *Oryza* and other grasses, and can be pathogenic causing rice blast disease (Ellis 1971).

95. *Ramichloridium schultzeri* (Sacc.) de Hoog.

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne*

Another hyphomycete fungus, *Ramichloridium schultzeri,* is characterised by its erect solitary dematiaceous condiophores which terminate with a rachis bearing small denticles from which sub globose conidia are produced. De Hoog and Hermanides-Nijhof, (1977) separated *R. schultzeri* into three sub species, based conidial and colony morphology and these root colonising isolates best fitted the description of *R. schultzeri* var. *schultzeri* de Hoog. It was isolated 32 times, 15 isolates from ryegrass and 17 isolates from white clover. It was more commonly obtained from Ruakura (24 isolates) than from Whatawhata (nine isolates). Hyphae were observed in the epidermis of all grass and legumes seedlings, and copious production of conidia was observed from conidiophores arising from infected roots. *Ramichloridium schultzeri* was pathogenic to sweet vernal seedlings, mildly pathogenic to red clover and ryegrass, and non pathogenic to; white clover, subterranean clover, lotus, Yorkshire fog, cocksfoot, browntop, timothy, soft brome and tall fescue. There is only one other report of it being pathogenic to roots (Nemec 1970), when it was found to be associated with strawberry root rot. Other reports of its occurrence are from cultivated soils, on wheat stems and leaves (Domsch *et al.* 1980).

96. *Rhizoctonia solani* Kuhn (=*Thanatephorous cucumeris* (Fank) Donk.)

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris*

One of the few basidiomycetes isolated from pasture roots was *R. solani.* A total of 24 isolates were obtained in the surveys. Although isolation frequency was relatively low, this fungus is an important and polyphagous plant root pathogen and further *in vitro* tests showed that it was pathogenic to all hosts tested. It caused complete seedling mortality of; cocksfoot, white clover, lotus, sweet vernal, timothy, browntop, and root lesions and necrosis to the remaining host species. It has been identified from over 40 plant species in

New Zealand (Pennycook 1989), which includes isolations from pasture species such as, white clover (Thornton 1965, Skipp and Watson 1987), perennial and Italian ryegrass (Falloon 1985, Skipp and Christensen 1989), *Poa* spp. (Christensen 1979), and browntop (Christensen 1979). *Rhizoctonia solani* was isolated from seedlings grown in Waikato soils and other North Island soils (Skipp and Watson 1987), whose results suggested this pathogen was more widespread in pastures than previous isolation frequencies indicated. It was also reported from New Zealand tussock-grassland soils at Waiouru and Alexandra (Morrison *et al.* 1959). In the USA, *R. solani* has been reported to cause damping off of seedlings and root rot of many pasture grasses and legumes (Sprague 1950, 1959, Farr *et al.* 1989), including all the pasture species sampled in this study. *Rhizoctonia* is subdivided into taxonomic groups based upon hyphal anastomosis, which is the a manifestation of somatic or vegetative incompatibility between isolates (Anderson, 1982) . Complete anastomosis is the fusion of hyphal walls of confronted isolates, but other reactions can occur such as the hyphal connection without fusion (Carling and Sumner 1992) . There are eleven anastomosis groups (AG) and host range and distribution differs for each (Carling and Sumner 1992), in addition each AG have been separated into sub groups based on morphology and pathogenicity (PG). The determination of AG and PG for the 24 isolates isolated in this study is required for further investigation of this important pasture root pathogen.

97. *Sordaria fimicola* (Rob.) Ces. and de Not.

Isolation site: Ruakura

Host: *Lolium perenne;*

A single isolate of this ascomycete was obtained from ryegrass. It is placed in the family Sordariaceae which is a genus characterised by smooth walled one-celled ascospores that are surrounded by a gelatinous sheath. *Sordaria fimicola* is one of the commonest coprophilous pyrenomycetes being isolated from the dung of wild and domesticated animals (Domsch *et al.* 1980), additionally it is isolated from soil and the rhizospheres of tea plants and tree species such as spruce. From *in vitro* tests, this fungus was found to colonise seedling roots of most grass and legume species tested and was non pathogenic.

98. *Sporothrix schenckii* Hektoen and Perkins

Isolation site: Whatawhata; Ruakura

Host: *Lolium perenne; Agrostis capillaris*

Two isolates of *S. schenckii* were found on ryegrass and browntop. It is rarely reported from roots but is common in soil and causes chronic subcutaneous mycoses in man.

Sterile fungi. (=Mycelia sterilia, Agonomycetes, Mitosporic fungi)

Sterile fungi are anamorphic mycelia which do not produce true conidia (Hawksworth *et al.*, 1995) . It is an artificial assemblage of sterile asexual mycelia which are classed as deuteromycetes (Fungi imperfecti). Although conidia are absent, non-dehiscent propagules (such as; allocysts, chlamydospores, bulbils and sclerotia) and similar structures (such as monilioid cells or hyphal swellings) may be produced in some genera of sterile fungi. Sterile fungi may be states of basidiomycetes, ascomycetes or other mitosporic fungi. There have been 28 genera comprising 200 species that have been officially described and classed as agonomycetes (Hawksworth *et al.* 1995).

The occurrence of sterile mycelium was a noticeable and important characteristic of the mycoflora pasture roots in this study, as total of 2824 sterile fungi were isolated, and this comprised almost 40% of all fungi obtained. Only four isolates were observed to have clamp connections indicating that most sterile fungi were probably ascomycetes or other mitosporic fungi. Sterile fungi were recorded on all pasture species at both sites and were therefore regarded as ubiquitous. Initially these isolates appeared to be a large amorphous group of fungi, however many had distinguishing cultural characteristics that allowed them to be separated into taxonomic or sterile groups (SG), the first of which was based on whether mycelium was hyaline or dematiaceous (99, 100 below). There is some discussion as to whether many fungi in culture are truly sterile (Hall 1985), as growing fungi in culture may not provide the required conditions to trigger production of asexual and sexual structures. For the purposes of this study any fungi not sporulating on standard laboratory media in the presence of near-UV light were recorded as being sterile (Hall 1985; Leach 1962) .

Sterile fungi are often reported to be isolated with high frequency from plant roots (Chu-Chou and Grace 1982, Hall 1985, Hall 1987, Singh 1980, Taylor and Parkinson 1964) and have been frequently reported from pasture plant roots (Davidson *et al.* 1977, Gadgil 1965, Waid 1957, Waid 1974). In New Zealand, both hyaline and dematiaceous forms are commonly recorded on; perennial ryegrass (Thornton 1965, Skipp and Christensen 1981, 1989, Falloon 1985, Sarathchandra *et al.* 1995), Italian ryegrass (Falloon 1985), white clover (Thornton 1965, Skipp and Christensen 1982, Skipp *et al.* 1982, Sarathchandra *et al.* 1995) and red clover (Skipp *et al.* 1986, Nan *et al.* 1991b). This study

therefore further confirms these non-sporing sterile forms as an important component of pasture root mycoflora.

99. Sterile dematiaceous (dark) fungi

A total of 2216 or 78% of sterile isolates were dematiaceous. From morphological studies nine distinct sterile dark groups were recognised, however a further 549 isolates could not be grouped and remained as an amorphous assemblage of dark sterile isolates. From *in vitro* tests all nine sterile groups were observed to penetrate epidermis and cortex tissues of most grass and legume hosts and several SG produced monilioid cells which were observed in roots tissues. Only two sterile dark groups produced conspicuous disease symptoms on seedling roots, the rest being assessed as non pathogenic.

100. Sterile hyaline fungi

The remaining 608 sterile isolates were hyaline with colorless or white mycelia, although one group produced orange mycelia on some media. Of these hyaline isolates, 393 cultures were able to be split into three taxonomic groups based on vegetative morphology. These three hyaline groups were non pathogenic to all tested grass and legume hosts and all were observed to penetrate the epidermis and cortex of all host seedlings.

101. *Tetracladium* De Wildem

Isolation site: Whatawhata
Host: *Trifolium repens*

Identified by its tetraradiate blastoconoidia, *Tetracladium* was recorded once on white clover which is a new host record. Conidia are distinctive consisting of a stalk surmounted by several branches in various arrangements. One branch is in line with the stalk and both constitute an axis. *Tetracladium* is an aquatic hyphomycete reported from ponds, streams and stream foam (Roldan *et al.* 1989) , and in New Zealand, species with *Tetracladium*-like conidia have also been described from streams (Aimer and Segedin 1985) . This genus has also been isolated from soils under grassland, from the roots of strawberry and gentians (Domsch *et al.* 1980), and *T. maxilliforme* (Rostrup) Ingold has been described as a leaf pathogen of clovers, producing sporodochia on lesions (Roldan *et al.* 1989). Cultural contamination of this single isolate meant it could not identified to species level before being discarded.

102. *Tetraploa aristata* Berk. and Br.

Isolation site: Ruakura

Host: *Trifolium repens*

A single isolate of this dematiaceous hyphomycete was isolated from clover. *Tetraploa aristata* was non pathogenic to grass and legume seedlings, but was it observed to cause root tip atrophy to white clover seedlings and to penetrate the root epidermal cells of; subterranean clover, white clover, red clover, ryegrass, sweet vernal, timothy, Yorkshire fog and cocksfoot. *Tetraploa aristata* is widespread usually found on leaf bases or stems at soil level and previous host records include; browntop, wheat, maize and other grass genera (Ellis 1971).

103. *Thielaviopsis basicola* (Berk. and Broome) Ferraris (synanamorph = *Chalara elegans* Nag Raj and Kendrick)

Isolation site: Ruakura

Host: *Trifolium repens*

Thielaviopsis basicola is a hyphomycete in the order Moniliales, with no known teleomorph. Nomenclature problems with this species have developed as a result of its pleomorphism and its close association with the ascomycete *Thielavia basicola* Zopf (Shew and Meyer 1992). On PCA it produces thick-walled pigmented chlamydospores (aleuriospores) and hyaline conidia from phialides, originally called endoconidia (Brierley, 1925). Nag Raj and Kendrick (1975) named the fungus *Chalara elegans*, and this name applies only to the endoconidial state. As the chlamydospore stage was observed on PCA it will be referred to as *T. basicola* in this study. Six isolates of *T. basicola* were exclusively isolated from white clover at Ruakura. This fungus has previously been isolated from white clover seedlings grown in Waikato soils (Skipp and Watson 1987), and these isolates were found to be pathogenic. From *in vitro* tests, this species was observed to colonise roots of all tested grass and legume seedlings and copious production of chlamydospores was often observed on the root surface and epidermis of seedlings. Systemic intercellular and intracellular penetration of this fungus was also previously reported in white clover roots (Lim and Cole 1984), where both hyphae and chlamydospores were observed to penetrate cortex cells and xylem vessels. It was assessed to be pathogenic to; red clover and sweet vernal, mildly pathogenic to; cocksfoot, tall fescue, subterranean clover, white clover, lotus, timothy and browntop, and non pathogenic to soft brome, ryegrass and Yorkshire fog. *Thielaviopsis basicola* is reported from all continents and is a facultative root parasite of numerous plants. It has a wide host range infecting over 150 plant species from 33 families but is particularly important on tobacco and legumes (Farr *et al.* 1989, Shew and Meyer 1992). It is also reported to colonise older or susceptible tissues of non hosts such as Poaceae (Gayed 1972) .

104. *Thozetella tocklaiensis* Agnihothrudu

Isolation site: Whatawhata

Host: *Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris*

Thozetella tocklaiensis was isolated 29 times from the roots of ryegrass, sweet vernal, and browntop which are new host records. Initially, isolates grew as sterile dematiaceous mycelium in culture but when grown on nutrient poor media under near-UV light, produced conidia in sporodochia characteristic of *T. tocklaiensis.* This fungus has not previously been reported in New Zealand and is unrecorded from plant roots. The fungus produced sporodochia on roots of grass and legume seedlings and hyphae penetrated epidermal and cortical root tissue of seedlings without causing visible disease symptoms. This fungus was first described by Agnihothrudu (1958), who isolated it from decaying floral parts of tea tree (*Camellia sinensis* L. O. Kuntze) in Assam, India. It was subsequently recorded from tea soils (Agnihothrudu 1962), and from leaf litter in Tanzania (Pirozynski 1972) . An unidentified species of *Thozetella* was reported from the litter of Japanese fir needles (Aoki and Tokumasu 1995) , and from dicotyledonous leaf litter in Brazil (Katz 1981) . This genus has therefore been primarily reported as a saprophyte of decayed plant litter and bark.

105. *Tolypocladium* W. Gams

Isolation site: Whatawhata

Host: *Agrostis capillaris*

Tolypocladium was isolated once from browntop. It is a soil-borne hyaline hyphomycete and is classed in the order Moniliales. It is characterised by floccose white colonies which produce one celled conidia in slimy heads from swollen phialides. It is also frequently reported to be an insect pathogen (Gams 1971b), and has rarely been recorded on plant roots, although several species have been isolated from alpine grassland soils (Bissett and Parkinson 1979a).

106. *Tricellula* van Beverw.

Isolation site: Whatawhata

Host: *Trifolium subterraneum*

Tricellula was isolated in survey three and all five isolates were obtained from subterranean clover which is a new host record. Colonies on PCA were slimy and pink reaching 10mm in diameter after seven days, and conidia were v-shaped and composed of three cells. These characteristics helped identify these isolates as *T. aquatica* Webster, but more morphological study is needed to confirm the species name. *Tricellula aquatica* is primarily an aquatic hyphomycete, but is also isolated from moist soils, wheat soils and

other agricultural soils (Domsch *et al.* 1980). In root colonisation and pathogenicity tests, it was assessed to be non pathogenic to all tested grasses and legumes, but hyphae were found penetrate epidermal cells of these grass and legume hosts. Species of *Tricellula* (*Rhyncosporium*) were common on the leaves of clover and perennial ryegrass in Waikato dairy pastures (di Menna and Parle 1970).

107. *Trichobotrys* Penzig and Saccardo

Isolation site: Whatawhata

Host: *Agrostis capillaris*

A single isolate of *Trichobotrys* was recorded from browntop roots which is a new host record and is also unrecorded on plant roots in New Zealand. Conidia were brown spherical and one celled. They were produced in chains, in succession towards the apex, from long cylindrical conidiophores which were slightly roughened, and this characteristic is similar to the type species *T. effusa* (Berk. and Broome) Petch, however, more morphological examination is needed to confirm the species name.

Trichoderma **Pers. ex Fr.**

The moniliaceous hyphomycete genus *Trichoderma*, is characterised by fast growing hyaline colonies bearing dendroid conidiophores in tufts and one-celled green (or hyaline) conidia. This genus was widespread on roots in all three surveys. A total of 373 *Trichoderma* isolates were obtained which comprised 5% of all fungi isolated, and six species were identified (tables 10 and 11). Similar mycological surveys in New Zealand, of the roots of perennial ryegrass and white clover, also report the frequent isolation of this genus (Thornton 1965, Skipp and Christensen 1982, 1989, Sarathchandra *et al.* 1995, Bonish unpublished). Gadgil (1965), additionally reported this genus from the roots of cocksfoot and meadowgrass, and Skipp (1994) from the roots of red clover. *Trichoderma* species have been reported to have both beneficial and deleterious affects in New Zealand pasture soils (Skipp 1994) . For example, *T. harzianum, T. koningii* and *T. pseudokoningii* were found to be antagonistic to plant pathogens, such as *Pythium irregulare* Buisman, reducing root rots on clovers and ryegrass. However all *Trichoderma* species tested, particularly *T. hamatum* and *T. polysporum,* were also pathogenic to pasture seedlings (Skipp 1994). Results from *in vitro* pathogenicity tests in this study also demonstrated some *Trichoderma* species were pathogenic to some host species and non pathogenic to other hosts (see entries 108-114). These *in vitro* tests may have been influenced by these species capacity to produce extracellular enzymes (Cairney and Burke 1994; Papavizas 1985), and therefore disease symptoms could be produced on roots without active invasion. Further evidence for this was that hyphal root penetration of some hosts was not observed (table 12), even though disease symptoms were observed.

Table 10. *Trichoderma* species isolated from roots in surveys one and two

Species	Ryegrass *	Clover *	Ryegrass	Clover	Browntop	Sweet Vernal	Total	%Total
T. hamatum	13	20	39	23	30	9	**134**	**39**
T. harzianum	14	15	4	5	2	5	**45**	**13**
T. koningii	19	20	10	2	5	2	**58**	**17**
T. polysporum	55	9	10	5	-	5	**84**	**24.5**
T. pseudokoningii	6	1	1	-	1	1	**10**	**3**
T. viride	2	-	-	1	-	-	**3**	**1**
Trichoderma sp.	3	-	2	-	4	-	**9**	**2.5**
Total	112	65	66	36	42	22	**343**	**100**

A total of 30 isolates of this genus were recorded in survey three which comprised 9% of all isolates recovered. *Trichoderma hamatum* was the most frequently isolated species of *Trichoderma* in survey three (Table 11). The smaller sample size of this survey probably accounted for the absence of *T. pseudokoningii* and *T. viride* from roots, and that only two isolates of the genus were recovered from lotus roots.

Table 11. *Trichoderma* species isolated from roots in survey three

SPECIES	Subterranean clover	Lotus	Yorkshire fog	Soft brome	Total	% Total
T. hamatum	2	2	-	7	**11**	**37**
T. harzianum	2	-	4	3	**9**	**30**
T. koningii	3	-	1	-	**4**	**13**
T. polysporum	-	-	3	3	**6**	**20**
Total	7	2	8	13	**30**	**100**

From the *in vitro* root colonisation tests, all tested *Trichoderma* isolates did not re-invade all seedlings (table 12). Of the hosts that were re-invaded, only intercellular invasion of epidermal cells was observed. Soft brome seedlings were resistant to recolonisation by any *Trichoderma,* and tall fescue was only colonised by the hyphae of *T. hamatum. Trichoderma viride* was not observed in of any seedlings, apart from white clover roots. These results indicate that isolates of *Trichoderma* are primarily colonisers of the root surface or epidermis of pasture seedlings.

Table 12. Root colonisation of pasture seedlings by *Trichoderma* species.

Species	WC*	SC	RC	LO	RG	TF	GG	TI	SV	BT	CF	YF
T. hamatum	1	1	1	1	0	1	0	1	1	1	1	1
T. harzianum	1	1	1	0	0	0	0	0	0	0	1	0
T. koningii	0	1	0	1	1	1	0	0	1	0	1	1
T. polysporum	1	0	1	1	1	0	0	0	1	1	1	1
T. viride	1	0	0	0	0	0	0	0	0	0	0	0

* WC white clover, SC subterranean clover, RC red clover, LO lotus, RG ryegrass, TF tall fescue, GG soft brome, TI timothy, SV sweet vernal, BT browntop, CF cocksfoot, YF Yorkshire fog.

108. *Trichoderma hamatum* (Bonord.) Bain

Isolation site: Whatawhata; Ruakura
Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Lotus uliginosus ; Trifolium subterraneum; Bromus hordeaceus.*

This species was the most common *Trichoderma* isolated, as 134 isolates were recorded from ryegrass, white clover, sweet vernal and browntop in surveys one and two, and 11 isolates were found on lotus, subterraneum clover and soft brome in survey three. It was observed to penetrate the epidermis of seedlings and was mildly pathogenic to; ryegrass, cocksfoot, red clover, subterranean clover, white clover, soft brome, timothy and browntop, and non pathogenic to; Yorkshire fog, lotus, tall fescue and sweet vernal. Domsch *et al.* (1980) reported this species to have a world wide distribution, but is the least commonly reported of the widespread *Trichoderma* species. This species was isolated from wheat and ryegrass roots and was found to be antagonistic to take-all fungus (*G. graminis* var. *tritici*), decreasing mortality of wheat and ryegrass plants inoculated with the pathogen (Dewan and Sivasithamparam 1988b) .

109. *Trichoderma harzianum* Rifai
Isolation site: Whatawhata; Ruakura
Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus; Trifolium subterraneum; Bromus hordeaceus.*

A total of 54 isolates of *T. harzianum* were identified in the study. This fungus is described as the smooth-spored counterpart to *T. viride* (Rifai 1969) . It is the most frequently received *Trichoderma* for identification at the International Mycological Institute (IMI), and is common in soil and rhizospheres of wheat, grasses, tobacco, pines and sugar beet (Domsch *et al.* 1980). Dewan and Sivasithsamparam (1988b) also recorded *T. harzianum* from the roots of ryegrass and wheat in Western Australia. In pathogenicity tests *T. harzianum* was observed in the epidermis of; subterranean clover, red clover, white clover, tall fescue and cocksfoot seedlings, and was pathogenic to these hosts causing root discolouration and small superficial lesions.

110. *Trichoderma koningii* Oudem.
Isolation site: Whatawhata; Ruakura
Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris; Holcus lanatus; Trifolium subterraneum.*

Trichoderma koningii was isolated a total of 62 times and was the third most frequently recorded *Trichoderma.* It was found to be pathogenic to only two of the tested host species; subterranean clover and lotus. In New Zealand, it has been previously identified from the roots of Brassicas (Falloon 1980a) and maize (Falloon 1982, Fowler 1985) and it was reported to be pathogenic to maize, perennial ryegrass, Italian ryegrass

(Falloon 1982, 1985). *Trichoderma* is one of the most studied genera of fungal antagonists, and *T. koningii* has frequently been reported to be antagonistic against many plant pathogens (Ghisalberti *et al.* 1990, Simon and Sivasithamparam 1989, Trutmann and Keane 1990). *Trichoderma koningii* was isolated from non surface sterilised roots of ryegrass and wheat roots in Western Australia, and like *T. hamatum,* it was found to be antagonistic to the take-all fungus.

111. *Trichoderma polysporum* (Link ex Pers.) Rifai

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Holcus lanatus; Bromus hordeaceus.*

Trichoderma polysporum was the second most frequently recorded species in this study with 90 isolates being identified, although no isolates were recorded from browntop roots. It has been previously reported on the roots of strawberry (Pennycook 1989), and from the rhizospheres of; wheat, lucerne, poplar and spruce (Domsch *et al.* 1980). It was pathogenic to seedlings of ryegrass, soft brome and red clover, and mildly pathogenic to; subterranean clover, lotus and tall fescue. Skipp (1994) also reported this species to be pathogenic to pasture seedlings, as lesions and root inhibition was observed on white clover, red clover and perennial ryegrass.

112. *Trichoderma pseudokoningii* Rifai

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris*

Only 10 isolates of *T. pseudokoningii* were identified, but it was still recorded at both sites and on all species sampled in survey one and two. Although it superficially resembles *T. koningii,* this fungus can be distinguished by different conidiophore branching pattern and production of a yellow pigment in culture. Like many other *Trichoderma* species, this species is frequently isolated from the soil and rhizosphere, and is antagonistic to many virulent root pathogens including *Rhizoctonia solani* (Hadwan and Khara 1992), *Phytophthora* (Chambers and Scott 1995), *Cylindrocladium scoparium* (Santos *et al.* 1993) and *Phymatotrichum omnivorum* (Kenerley *et al.* 1987).

113. *Trichoderma viride* Pers. ex Gray

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne*

This species was rarely recorded from pasture roots, as only three isolates were identified by roughened spherical conidia. It was not pathogenic to most seedlings, apart from those of red clover, which became discoloured after inoculation, in addition it was

only observed in the root epidermis of white clover. It was reported to inhibit germination and root growth of perennial ryegrass seedlings (Humphreys-Jones and Waid 1963), which was not observed in these similar pathogenicity tests. *Trichoderma viride* is one of the most widespread soil fungi and was the commonest fungi isolated from Dutch agricultural soils (Gams 1992). Although it was uncommon in this study, it has been recorded from the roots of numerous plant hosts ranging from Beech to pineapple (Domsch *et al.* 1980). Waid (1974) reported that *T. viride* is more frequently isolated from soils than from plant roots growing in the same soils.

114. *Trichosporon cutaneum* (De Beurm. *et al.*)

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris*

Trichosporon cutaneum was the most frequently isolated yeast in this study with 47 isolates being recorded in survey one and two. It was identified by its nitrogen and carbon source utilisation (Kreger-van Rij 1984), and its morphology, producing arthroconidia and blastoconidia in culture. There are few reports of this fungus from plant roots, however it is a common saprophyte of in soil, fresh and seawater, and plant detritus (Domsch *et al.* 1980). *Trichosporon cutaneum* was also commonly isolated from many New Zealand soils (di Menna 1965, di Menna 1968) . From *in vitro* tests it was assessed to be non pathogenic to pasture seedlings but was able to penetrate the epidermis of most hosts species apart from soft brome, timothy and tall fescue.

115. *Verticicladiella* S. Hughes

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris*

This fungus was infrequently encountered with only 13 isolates being recorded, but was present on roots at both sites from all four pasture species sampled in survey one and two. It was found to recolonise the epidermis of all tested hosts and there was copious production of conidiophores on the agar root-surface interface. It was assessed to be non pathogenic to all seedlings as no conspicuous root damage was seen. This genus has not been previously reported from pasture roots in New Zealand, but *V. truncata* Wingfield and Marasas and *V. procera* Kendrick have been recorded from Pine roots (Pennycook 1989).

***Verticillium* Nees von Esenbeck**

The genus *Verticillium* is based on conidiophore morphology, and consists over 40 species (Melouk 1992). Conidia are borne singly or in clusters on conidiophores which are septate, erect and branched, with short branches forming whorls. The genus is regarded as an artificial grouping of often unrelated species that needs taxonomic revision (Williams

pers. comm.), as solely basing the classification on conidiophore morphology has proved unsatisfactory for some species. There has been some revision with Gams and Van Zaayen (1982) dividing the genus into four sections. Five species were recorded from pasture roots (see 116- 120 below) with a total of 90 isolates (1.2%) being obtained. This genus contains vascular and non vascular parasites of plants that are of great economic importance (Melouk 1992). *Verticillium* species are also common clover leaf inhabitants in New Zealand (di Menna and Parle 1970). In the USA this genus has been reported to cause root necrosis of pasture legumes such as red clover and lotus (Farr *et al.* 1989).

116. *Verticillium albo-atrum* Reinke and Berthold

Isolation site: Whatawhata

Host: *Trifolium repens*

A single isolate of *V. albo-atrum* was identified from clover. This species is similar to *V. dahliae,* but is distinguished by swollen darkly pigmented hyphae and the absence of microsclerotia. This species has a very wide host range and is commonly reported from cultivated plants such as clover, cotton and tomato (Pennycook 1989, Melouk 1992). This fungus has been reported to be an important pathogen of lucerne in New Zealand pastoral agriculture (Hawthorne 1983). *Verticillium albo-atrum* is reported to have a weak competitive ability in soil (Born 1971) as it is a strict root inhabiting fungus and its hyphae do not spread far beyond roots into the soil (Isaac 1953, Isaac 1967) .

117. *Verticillium cephalosporum* W. Gams

Isolation site: Whatawhata

Host: *Agrostis capillaris*

This fungus was isolated once from browntop in survey one. There are no reported records of this fungus being isolated from browntop roots or any other plant roots, but a closely related species, *V. psallotiae* Treschow, has been recorded on ryegrass roots (Labruyere 1979).

118. *Verticillium chlamydosporium* Goddard (= *Diheterospora chlamydosporia* (Goddard) Barron and Onions).

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Anthoxanthum odoratum; Agrostis capillaris*

Verticillium chlamydosporium was recorded 44 times from ryegrass, white clover , sweet vernal and browntop roots, making it the most common species of *Verticillium* to be isolated. It was also observed to penetrate the epidermis of all inoculated grass and legume seedlings apart from soft brome, and was non pathogenic to all tested host plants. *V. chlamydosporium* has been reported from forest, grassland, arable, and garden soils

(Domsch *et al.* 1980). In New Zealand it has previously reported in pastures on the roots of perennial ryegrass and white clover (Thornton 1965, Skipp and Christensen 1989), and it was frequently isolated from nematode (*Heterodera trifolii* Goffart) cysts in North Island pastures (Hay and Skipp 1993). This fungus is both nematophagous and colonises plant roots (Bourne *et al.* 1994) so has been investigated as a biocontrol agent against plant parasitic nematodes (Kerry *et al.* 1993, Kerry *et al.* 1984) .

119. *Verticillium dahliae* Kleb.

Isolation site: Whatawhata; Ruakura

Host: *Trifolium repens; Lolium perenne; Agrostis capillaris*

This fungus is a widespread plant wilt pathogen was isolated 41 times from Waikato pastures. It is similar to *V. albo-atrum,* in that it is a strict root fungus with little growth into the soil (Isaac 1967), and has a large host range, being reported from over 100 plant species in New Zealand alone (Pennycook 1989). In New Zealand pastures, it has been previously isolated from red clover (Nan *et al.* 1991a, Skipp 1986), on which it is also pathogenic.

120. *Verticillium lecanii* (Zimm.) Viegas

Isolation site: Whatawhata

Host: *Trifolium subterraneum*

Three isolates of *V. lecanii* were obtained from subterranean clover roots in survey three which is a new host record as it has rarely been reported from roots. This fungus has been recorded on barley seedlings, uncultivated soils, tundra soils, grassland soils and leaf litter (Domsch *et al.* 1980) but is also one of the most important entomogenous hyphomycetes occurring on many insect species from all climates.

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APPENDIX 4: Average colony growth rates of fungi inoculated onto PCA and incubated at 20°C for 7 days.

FUNGAL SPECIES:	FAST	MEDIUM	SLOW
	>50mm	20-50mm	<20mm
Acremoniella atra		24.8	
Acremonium curvulum		34.1	
Acremonium fusidioides		27.7	
Acremonium kiliense		24.5	
Acremonium murorum		23.7	
Acremonium sp.		22.9	
Acremonium strictum			19.0
Alternaria alternata		40.2	
Aphanocladium album		46.8	
Arthrinium arundinis	53.2		
Arthrobotrys oligospora		25.0	
Aspergillus fumigatus		21.0	
Aspergillus niger		23.7	
Aspergillus ustus			17.9
Aureobasidium pullulans			15.3
Beauveria bassiana		26.7	
Bimuria novae-zelandiae			14.1
Bipolaris sp.		30.3	
Botrytis cinerea	80+		
Botryosporium	52.4		
Chaetomium funicola			17.5
Chaetomium globosum			18.9
Chaetomium indicum			16.0
Chrysosporium		35.9	
Cladosporium herbarum		29.0	
Clasterosporium sp.			14.5
Codinaea fertilis		44.0	
Colletotrichum sp.		39.0	
Curvularia trifolii		22.4	
Cylindrocarpon destructans	56.2		
Cylindrocladium scoparium	80+		
Dactylaria acerosa			9.5
Dreschlera dematioidea		28.8	
Epicoccum nigrum	60.4		
Fusarium acuminatum	58.4		
Fusarium avenaceum	78.6		
Fusarium crookwellense	80+		
Fusarium culmorum	80+		
Fusarium equiseti	57.5		
Fusarium graminum	51.2		
Fusarium oxysporum	80+		
Fusarium sambucinum	77.4		
Fusarium solani	69.0		
Fusarium tricinctum	80+		

FUNGAL SPECIES:	FAST	MEDIUM	SLOW
Geotrichum candidum			15.6
Gliocladium roseum		40.0	
Gliocladium sp 1		38.4	
Gliocladium sp 2		24.6	
Gongronella butleri	79.0		
Humicola fuscoatra			17.6
Idriella bolleyi		25.0	
Idriella lunata		21.3	
Mariannaea elegans			15.8
Metarhizium anisopliae			16.2
Mortierella alpina	76.5		
Mortierella bainerí	67.4		
Mortierella elongata	70.8		
Mortierella gamsii	80		
Mortierella globulifera	69.5		
Mortierella hyalina	78.4		
Mucor sp.	80+		
Myrothecium verrucaria		36.2	
Paecilomyces carneus			10.3
Paecilomyces lilacinus		28.9	
Paecilomyces marquandii			17.5
Penicillium atramentosum		21.7	
Penicillium brevicompactum			11.3
Penicillium chrysogenum		22.7	
Penicillium citrinum			10.8
Penicillium crustosum			13.3
Penicillium decumbens			13.0
Penicillium dendriticum			15.1
Penicillium expansum			17.5
Penicillium glabrum			8.9
Penicillium griseofulvum			12.9
Penicillium islandicum			9.7
Penicillium janczewskii			7.9
Penicillium janthinellum		25.0	
Penicillium minioluteum			10.4
Penicillium oxalicum		22.4	
Penicillium raistrickii			8.8
Penicillium rugulosum			15.6
Penicillium simplicissimum		24.7	
Penicillium sp.(*wacksmannii*)			15.0
Penicillium variabile			9.5
Periconia macrospinosa		45.7	
Pestalotia		23.7	
Phymatotrichum omnivorum	80+		
Pithomyces chartarum		29.0	
Plectosporium tabacinum	58.0		
Preussia aemulans			14.0
Pseudallescheria boydii		30.5	

FUNGAL SPECIES:	FAST	MEDIUM	SLOW
Pycnidial fungi*	F	M	S
Pyrenophora		24.1	
Pyricularia oryzae			13.8
Ramichloridium schultzeri			19.3
Rhizoctonia solani	80+		
Sporothrix schenckii	75.1		
Sterile dark fungi*	F (50-80+)	M (20-49)	S (10-19)
Sterile dark group 1	55.0		
Sterile dark group 2			
Sterile dark group 3			6.0
Sterile dark group 4	70		
Sterile dark group 5		45-55	
Sterile dark group 6	70-80+		
Sterile dark group 7			15.5
Sterile dark group 8			5.0
Sterile dark group 9		35	
Sterile hyaline fungi*	F(50-80+)	M (20-49)	S (5-19)
Sterile hyaline group 1			8.0
Sterile hyaline group 2	80+		
Sterile hyaline group 3			10.0
Tetracladium **sp.**			16.2
Tetraploa aristata			7.4
Thielaviopsis basicola		23.4	
Thozetella tocklaiensis			17.0
Tolypocladium	55.9		
Trichobotrys **sp.**		41.3	
Tricellula			10.0
Trichoderma hamatum	80+		
Trichoderma harzianum	80+		
Trichoderma koningii	80+		
Trichoderma polysporum	80+		
Trichoderma psuedokoningii	80+		
Trichoderma **sp.**	80+		
Trichoderma viride	80+		
Trichosporon cutaneum			13.0
Verticicladiella **sp.**			15..5
Verticillium albo-atrum		27.0	
Verticillium cephalosporum	50.1		
Verticillium chlamydosporium		38.5	
Verticillium dahliae		45.4	
Verticillium lecanii		31.0	
TOTAL	**2524**	**3544**	**1121**

80+ = average colony growth reached the edge of the plate within 7 days.

* Pycnidial fungi, sterile dark fungi and sterile hyaline fungi were an amorphous group of many species which comprised fast, medium and slow isolates.

APPENDIX 5: Number and frequency of fungi isolated from North and South facing pasture slopes at Whatawhata in survey one.

Aspect	Northern slopes		Southern slopes		Survey one Total	
Fungal Species	**Count**	**%**	**Count**	**%**	**Total**	**%**
Acremoniella atra	2	0.10	-	-	2	0.05
Acremonium curvulum	15	0.77	15	0.72	30	0.75
Acremonium fusidioides	-	-	14	0.67	14	0.35
Acremonium kiliense	5	0.26	15	0.72	20	0.50
Acremonium murorum	5	0.26	-	-	5	0.12
Acremonium sp.	2	0.10	4	0.19	6	0.15
Acremonium strictum	1	0.05	2	0.10	3	0.07
Alternaria alternata	2	0.10	2	0.10	4	0.10
Aphanocladium album	7	0.36	1	0.05	8	0.20
Arthrinium arundinis	2	0.10	7	0.34	9	0.22
Arthrobotrys oligospora	2	0.10	3	0.14	5	0.12
Aspergillus fumigatus	-	-	1	0.05	1	0.02
Aspergillus niger	-	-	4	0.19	4	0.10
Aureobasidium pullulans	5	0.26	7	0.34	12	0.30
Beauveria bassiana	-	-	1	0.05	1	0.02
Binuria novae-zelandiae	11	0.57	8	0.38	19	0.47
Bipolaris sp.	1	0.05	-	-	1	0.02
Botrytis cinerea	4	0.21	3	0.14	7	0.17
Chaetomium funicola	13	0.67	10	0.48	23	0.57
Chaetomium globosum	5	0.26	-	-	5	0.12
Chaetomium indicum	1	0.05	-	-	1	0.02
Chrysosporium	-	-	1	0.05	1	0.02
Cladosporium herbarum	7	0.36	14	0.67	21	0.52
Clasterosporium spp.	3	0.15	-	-	3	0.07
Codinaea fertilis	179	9.21	151	7.27	330	8.20
Colletotrichum sp.	3	0.15	13	0.63	16	0.40
Curvularia trifolii	1	0.05	2	0.010	3	0.07
Cylindrocarpon destructans	23	1.18	25	1.20	48	1.19
Cylindrocladium scoparium	2	0.10	29	1.40	31	0.77
Dactylaria acerosa	39	2.01	28	1.35	67	1.67
Dreschlera dematioidea	6	0.31	5	0.24	11	0.27
Epicoccum nigrum	5	0.26	2	0.10	7	0.17
Fusarium acuminatum	3	0.15	6	0.29	9	0.22
Fusarium avanaceum	25	1.29	14	0.67	39	0.97
Fusarium crookwellense	19	0.98	20	0.96	39	0.97
Fusarium culmorum	4	0.21	5	0.24	9	0.22
Fusarium equiseti	-	-	1	0.05	1	0.02
Fusarium graminum	-	-	2	0.01	2	0.05
Fusarium oxysporum	263	13.53	126	6.06	389	9.67
Fusarium sambucinum	-	-	1	0.05	1	0.02
Fusarium solani	8	0.41	4	0.19	12	0.30
Fusarium tricinctum	-	-	2	0.10	2	0.05
Geotrichum candidum	1	0.05	-	-	1	0.02
Gliocladium roseum	43	2.21	56	2.29	99	2.46
Gliocladium sp 1	-	-	1	0.05	1	0.02
Gliocladium sp 2	1	0.05	-	-	1	0.02
Gongronella butleri	8	0.41	6	0.29	14	0.35
Idriella bolleyi	4	0.21	12	0.58	16	0.40

Aspect	Northern slopes		Southern slopes		Survey one Total	
Fungal Species	**Count**	**%**	**Count**	**%**	**Total**	**%**
Idriella lunata	-	-	1	0.05	1	0.02
Mariannaea elegans	9	0.46	10	0.48	19	0.47
Metarhizium anisopliae	2	0.10	-	-	2	0.05
Mortierella alpina	-	-	3	0.14	3	0.07
Mortierella elongata	2	0.10	4	0.19	6	0.15
Mortierella gamsii	3	0.15	6	0.29	9	0.22
Mortierella globulifera	23	1.18	7	0.34	30	0.75
Mortierella hyalina	-	-	1	0.05	1	0.02
Mucor spp.	5	0.26	8	0.38	13	0.32
Myrothecium verrucaria	5	0.26	5	0.24	10	0.25
Paecilomyces carneus	12	0.62	16	0.77	28	0.70
Paecilomyces lilacinus	30	1.54	9	0.77	39	0.97
Paecilomyces marquandii	1	0.05	2	0.10	3	0.07
Penicillium atramentosum	3	0.15	1	0.05	4	0.10
Penicillium brevicompactum	6	0.31	2	0.10	8	0.20
Penicillium chrysogenum	15	0.77	4	0.19	19	0.47
Penicillium citrinum	2	0.10	-	-	2	0.05
Penicillium decumbens	1	0.05	-	-	1	0.02
Penicillium islandicum	-	-	1	0.05	1	0.02
Penicillium janczewskii	1	0.05	-	-	1	0.02
Penicillium janthinellum	34	1.75	35	1.68	69	1.72
Penicillium minioluteum	1	0.05			1	0.02
Penicillium simplicissimum	17	0.87	32	1.54	49	1.22
Penicillium spp.	-	-	6	0.29	6	0.15
Penicillium variabile	1	0.05	-	-	1	0.02
Periconia macrospinosa	34	1.75	17	0.82	51	1.27
Pithomyces chartarum	3	0.15	-	-	3	0.07
Plectosporium tabacinum	6	0.31	7	0.34	13	0.32
Preussia aemulans	-	-	6	0.29	6	0.15
Pseudallescheria boydii	1	0.05	-	-	1	0.02
Pycnidial fungi	27	1.39	33	1.59	60	1.49
Pyricularia oryzae	4	0.21	2	0.10	6	0.15
Ramichloridium schultzeri	1	0.05	7	0.34	8	0.20
Rhizoctonia solani	10	0.51	12	0.58	22	0.35
Sporothrix schenckii	-	-	1	0.05	1	0.02
Sterile dark fungi	112	5.76	182	8.76	294	7.31
Sterile dark group 1	7	0.36	15	0.72	22	0.55
Sterile dark group 2	-	-	1	0.05	2	0
Sterile dark group 3	78	4.22	66	2.98	144	1.23
Sterile dark group 4	42	2.16	110	5.29	152	3.78
Sterile dark group 5	197	10.13	157	7.56	354	8.80
Sterile dark group 6	101	5.20	169	8.13	270	6.71
Sterile dark group 7	72	3.70	87	4.19	159	3.95
Sterile dark group 8	23	1.18	23	1.11	47	0.68
Sterile hyaline fungi	54	2.78	66	3.18	120	2.98
Sterile hyaline group 1	20	1.03	28	1.35	48	1.19
Sterile hyaline group 2	4	0.21	-	-	4	0.10
Sterile hyaline group 3	92	4.73	161	7.75	282	4.09
Tetracladium sp.	1	0.05	-	-	1	0.02

Thozetella tocklaiensis	11	0.57	18	0.87	29	0.72
Tolypocladium sp.	-	-	1	0.05	1	0.02
Trichobotrys sp.	-	-	1	0.05	1	0.02
Trichoderma hamatum	51	2.62	50	2.41	101	2.51
Trichoderma harzianum	12	0.62	4	0.19	16	0.40
Trichoderma koningii	5	0.26	14	0.67	19	0.47
Trichoderma polysporum	5	0.26	15	0.72	20	0.50
Trichoderma psuedokoningii	2	0.10	1	0.05	3	0.07
Trichoderma sp.	5	0.26	1	0.05	7	0.17
Trichosporon cutaneum	25	1.29	12	0.58	37	0.92
Unidentified species	13	0.67	2	0.10	15	0.37
Verticicladiella sp.	1	0.05	5	0.24	6	0.15
Verticillium albo-atrum	1	0.05	-	-	1	0.02
Verticillium cephalosporum	-	-	1	0.05	1	0.02
Verticillium chlamydosporium	10	0.51	9	0.43	11	0.27
Verticillium dahliae	12	0.62	15	0.72	27	0.67
TOTAL	**1945**	**100**	**2078**	**100**	**4023**	**100**
No. of species identified	**77**		**83**		**100**	

Appendix 6 Constituents for buffers and stains required for electrophoresis and isozyme analysis

Phosphate buffer

NaH_2PO_4	0.384g
Na_2HPO_4	2.386g
2-mercaptoethanol	0.025 ml
distilled water	100 ml

Buffer Systems used for electrophoresis, from protocols outlined by Soltis *et al.* 1983, Micales *et al.* 1986, Wendel and Weeden 1989.

Amine-citrate (AC), pH 6.1,run at 170 v

Ridgeway buffer (RW), pH 8.5, run at 250v,

Poulik (discontinuous tris-citrate) (PK), pH 5.7, run at 300v

Tris-citrate (TC), pH 7.1, run at 100v

Phosphate (PH), pH 7.5, run at 150 v

APPENDIX 7:

I. A preliminary pathogenicity screen of fungal isolates to determine within strain variability of 20 fungal species to perennial ryegrass and white clover.

Before the 70 species of fungi selected were screened for pathogenicity to a selection of common pasture legumes and grasses it was necessary to determine if there was any strain variability within a sample of isolates of these fungi.

Methods

Six different isolates (or strains) from twenty species of fungi (Table 1) were chosen to be inoculated onto axenically grown seedlings. The same rapid *in vitro* screening technique outlined in the methods of chapter three, was used to determine pathogenicity and root colonisation of each fungus.

The same disease scores used in chapter three (0-5) were assigned to each seedling based on observable disease symptoms. A mean disease score was then calculated for each host by averaging all six disease scores for each host plant. A root segment from each inoculated seedling was plated onto WA to reisolate the fungus from diseased tissue. A pathogenicity rating was calculated for each isolate on each host by averaging the six disease scores from plates for each treatment.

Statistical analysis: All data were statistically analysed using analysis of variance and LSD tests were used for mean separation between isolates.

Table 1. Isolates used to test strain variability of pathogenicity to perennial ryegrass and white clover

Fungal species	Strain/Isolate	Original host
Bimuria novae zelandiae	1. C1R6.1P28S5*	White clover
	2. C1WN5P3S7	White clover
	3. C1WN5P14S11	White clover
	4. C2R4.3P1S5	White clover
	5. C2R4.3P9S12	White clover
	6. C2WNA3P2S5	White clover
Codinaea fertilis	1. C2WS10P3S4	White clover
	2. R2R6.1P10S3	Ryegrass
	3. CIR6.1P25S5C*	White clover
	4. C2R4.3P11S2	White clover
	5. SV1WN1P5S7	Sweet vernal
	6. B1WN5P9S3	Browntop
Cylindrocarpon destructans	1. R1R4.3P21S2	Ryegrass
	2. R1R6.2P17S1*	Ryegrass
	3. R1R6.2P20S7	Ryegrass
	4. C2WNA3P2S4	White clover
	5. B1WS2R3P1S1	Browntop
	6. R1R6.2P17S1	Ryegrass
Cylindrocladium scoparium	1. C2R4.2P15S15*	White clover
	2. SCWNS3	Subterranean clover
	3. R1R6.2P5S5	Ryegrass
	4. C2WS7P8S1	White clover
	5. C2WS1P1S2	White clover
	6. C2WS1P7S4	White clover

Fungal species	Strain/Isolate	Original host
Fusarium acuminatum	1. B2WS2P9S1	Browntop
	2. R2WS3P7S3A	Ryegrass
	3. R1R4.3P16S3	Ryegrass
	4. C1WTOPP7S7	White clover
	5. SV1WN3P2S1*	Sweet vernal
	6. R1WN2.4P8S5	Ryegrass
Fusarium avenaceum	1. C1R4.3P15S2	White clover
	2. SVWNA2P11S2*	Sweet vernal
	3. R1WN5P1S2	Ryegrass
	4. SV1WS3P10S4	Sweet vernal
	5. YFWS6	Yorkshire fog
	6. B2WSB1P8S1	Browntop
Fusarium crookwellense	1. C2WNB1P4S2*	White clover
	2. R2WNB1P1S3	Ryegrass
	3. R2WNA2P1S5A	Ryegrass
	4. LWSS4	Lotus
	5. R1R4.3P3S4	Ryegrass
	6. B2WNA3P3S1	Browntop
Fusarium culmorum	1. R2WS7P4S2A	Ryegrass
	2. LWNS18	Lotus
	3. R1R4.3P21S7*	Ryegrass
	4. LWNS20	Lotus
	5. B2WNA1P6S5	Browntop
	6. R2R4.3P7S2	Ryegrass
Fusarium oxysporum	1. R2R6.2P3S1	Ryegrass
	2. R2WNA1P5S7*	Ryegrass
	3. SV2WNA1P1S1	Sweet vernal
	4. SV2WS1P5S1	Sweet vernal
	5. C2WNA3P6S3	White clover
	6. B2WS2P3S1	Browntop
Fusarium solani	1. R1R4.2P12S12	Ryegrass
	2. B2WNB1P6S1	Browntop
	3. R2WNB1P3S5	Ryegrass
	4. C2R6.2P3S1	White clover
	5. C2WNB1P7S3	White clover
	6. R1R4.2P12S12*	Ryegrass
Gongronella butleri	1. C2R4.2P3S2	White clover
	2. B2WNB1P6S6	Browntop
	3. LWNS7	Lotus
	4. SCWNS10	Subterranean clover
	5. R1R6.1P16S7B	Ryegrass
	6. R2R4.2P7S1*	Ryegrass
Periconia macrospinosa	1. R2WNA3P3S2	Ryegrass
	2. SVWNA1P4S1*	Sweet vernal
	3. C1R4.3P24S6	White clover
	4. SV2WS3P2S6	Sweet vernal
	5. YFWNS37	Yorkshire fog
	6. GGWNS28	Soft brome

Fungal species	Strain/Isolate	Original host
Paecilomyces carneus	1. B2WNA2P3S4	Browntop
	2. C2WSB1P10S1	White clover
	3. SV2WSBP10S1C*	Sweet vernal
	4. B2WS2P4S2	Browntop
	5. YFWSS31	Yorkshire fog
	6. R1WS1P1S8	Ryegrass
Sterile Dark Group 1	1. R1R6.1P18S5	Ryegrass
	2. R1R4.2P10S7	Ryegrass
	3. SV1WS4P4S8	Sweet vernal
	4. SV2WS1P4S4	Sweet vernal
	5. C2WNA1P4S5*	White clover
	6. R2WSB1P2S4B	Ryegrass
Sterile Dark Group 5	1. R2R4.2P8S4	Ryegrass
	2. C1WN5P9S7*	White clover
	3. B1WN5P1S6	Browntop
	4. C1WN3P5S3	White clover
	5. C1R4.2P1S9	White clover
	6. SV1W1.2R1P8S7	Sweet vernal
Sterile Dark Group 6	1. SV1W1R3P8S6*	Sweet vernal
	2. R1WS3P7S3	Ryegrass
	3. C1R4.2P14S5	White clover
	4. B1WN2.1P4S10	Browntop
	5. R2WS7P10S7	Ryegrass
	6. B2WSB1P1S4	Browntop
Sterile Hyaline Group 1	1. SV2WNB1P1S2	Sweet vernal
	2. C1WS9P1S4*	White clover
	3. SV1W1.2R1P7S6	Sweet vernal
	4. SV1W1R2P8S4	Sweet vernal
	5. R1R6.1P13S2	Ryegrass
	6. C1R4.2P26S5A	White clover
Sterile Hyaline Group 3	1. B1WS2.7P1S9	Browntop
	2. C1WS7P5S9	White clover
	3. B1WN2.3P5S9	Browntop
	4. B1WS2R2P2S10*	Browntop
	5. SV1WS3P4S10	Sweet vernal
	6. R1WS7P5S1	Ryegrass
Thozetella tocklaiensis	1. B2WNA2P2S4*	Browntop
	2. SV2WS1P6S1	Sweet vernal
	3. B2WNA2P1S4	Browntop
	4. B2WNB1P4S1	Browntop
	5. SV2WNB1P7S2	Sweet vernal
	6. R2WNB1P1S2	Ryegrass
Verticillium chlamydosporium	1. SVWNA3P14S5*	Sweet vernal
	2. R2WS3P2S5	Ryegrass
	3. B2WNB1P6S4B	Browntop
	4. C2R4.3P8S3	White clover
	5. C2WSB1P6S5	White clover
	6. SVWS1P9S4	Sweet vernal

* indicates this strain was used in the pathogenicity screen outlined in chapter three.

Results

Eight fungi, *C. fertilis, C. destructans, C. scoparium, F. acuminatum, F. avenaceum, F. crookwellense, F. culmorum* and *F. solani,* were found to be pathogenic to white clover seedlings (Table 2), as all had disease scores greater than 3. The remainder of fungi had low mean disease scores and were therefore non pathogenic.

Table 2. Mean disease scores of white clover seedlings inoculated with six strains each of 20 fungal species

	Mean Disease Scores						TOTAL
Fungal Species	**Strain 1**	**Strain 2**	**Strain 3**	**Strain 4**	**Strain 5**	**Strain 6**	**MEAN**
Bimuria novae zelandiae	1.7	1.8	1.4	1.7	0.8	1.1	**1.40**
Codinaea fertilis	3.2	2.8	3.3	3.5	2.9	2.9	**3.11***
Cylindrocarpon destructans	2.5	3.3	3.1	3.2	3.1	3.2	**3.07***
Cylindrocladium scoparium	5.0	5.0	4.8	5.0	4.9	5.0	**4.95***
Fusarium acuminatum	4.1	4.2	4.6	3.9	4.7	4.4	**4.32***
Fusarium avenaceum	4.9	3.9	5.0	5.0	5.0	4.9	**4.81***
Fusarium crookwellense	5.0	4.9	5.0	4.9	4.9	5.0	**4.96**
Fusarium culmorum	5.0	5.0	4.9	5.0	4.9	5.0	**4.97***
Fusarium oxysporum	1.8	4.2	2.1	2.2	2.3	4.0	**2.76**
Fusarium solani	3.7	3.9	4.2	3.4	3.7	4.1	**3.85***
Gongronella butleri	0.4	0.5	0.1	0.2	0.1	0.2	**0.25**
Paecilomyces carneus	0.4	0.2	0.6	0.5	0.3	0.5	**0.41**
Periconia macrospinosa	2.5	2.3	2.6	2.7	2.8	2.8	**2.63**
Sterile Dark Group 1	0.4	0.7	0.3	0.2	0.4	0.2	**0.35**
Sterile Dark Group 5	1.6	1.8	1.8	1.6	1.6	2.0	**1.75**
Sterile Dark Group 6	2.5	2.8	2.8	3.1	2.8	2.8	**2.85**
Sterile Hyaline Group 1	0.2	0.2	0.1	0.2	0.1	0.3	**0.17**
Sterile Hyaline Group 3	0	0.2	0.2	0	0.2	0	**0.09**
Thozetella tocklaiensis	0.6	0.2	0.6	0.4	0.5	0.8	**0.51**
Verticillium chlamydosporium	0.4	0.2	0.1	0.4	0.1	0.1	**0.21**
CONTROL	0.1	0.2	0	0	0.1	0.3	**0.11**

*** Total mean disease scores of all 6 strains is larger than 3 and are therefore considered to be pathogenic.**

Nine fungi, *C. fertilis, C. destructans, C. scoparium, F. acuminatum, F. avenaceum, F. crookwellense, F. culmorum, F. oxysporum,* and *F. solani,* were found to be pathogenic to ryegrass seedlings (Table 3) as all had disease scores >3. The remainder of fungi had low mean disease scores <3 and were therefore classed as weakly to non pathogenic.

Table 3 Mean disease scores of ryegrass seedlings inoculated with six strains each of 20 fungal species.

	Mean Disease Scores						TOTAL
Fungal Species	**Strain 1**	**Strain 2**	**Strain 3**	**Strain 4**	**Strain 5**	**Strain 6**	**MEAN**
Bimuria novae zelandiae	0.2	0	0.4	0.9	0.3	0.4	**0.38**
Codinaea fertilis	3.2	3.3	3.3	3.0	2.9	3.2	**3.13***
Cylindrocarpon destructans	3.8	4.0	3.8	3.7	3.7	3.6	**3.75***
Cylindrocladium scoparium	5.0	4.9	5.0	4.8	4.9	5.0	**4.94***
Fusarium acuminatum	3.9	4.4	3.9	4.4	4.0	4.1	**4.13***
Fusarium avenaceum	5.0	5.0	5.0	4.4	5.0	5.0	**4.89***
Fusarium crookwellense	4.7	4.9	5.0	5.0	4.8	4.7	**4.85***
Fusarium culmorum	4.6	4.7	4.8	4.7	4.9	4.9	**4.76***
Fusarium oxysporum	1.8	3.5	3.2	2.6	2.8	4.1	**3.00***
Fusarium solani	2.8	3.9	1.3	3.0	3.9	3.9	**3.14***
Gongronella butleri	0.1	0.3	0.3	0.2	0.4	0.2	**0.23**
Paecilomyces carneus	0.2	0.1	0.3	0.5	0	0.6	**0.28**
Periconia macrospinosa	2.3	2.6	2.2	2.0	2.7	1.9	**2.29**
Sterile Dark Group 1	0.1	0.7	0.2	0.4	0.2	0.4	**0.32**
Sterile Dark Group 5	1.1	1.3	0.9	1.7	1.3	1.4	**1.29**
Sterile Dark Group 6	2.7	1.9	1.9	2.6	1.7	2.1	**2.15**
Sterile Hyaline Group 1	0.3	0	0.3	0.2	0.2	0.3	**0.22**
Sterile Hyaline Group 3	0	0.2	0.3	0.3	0.3	0.1	**0.21**
Thozetella tocklaiensis	0.2	0.2	0.7	0.7	0.2	0.4	**0.42**
Verticillium chlamydosporium	0.1	0	0.2	0.3	0.3	0.1	**0.16**
CONTROL	0.4	0.1	0.1	0.1	0.3	0.2	**0.19**

*** Total mean disease scores of all 6 strains is larger than 3 and are therefore considered to be pathogenic.**

There was a highly significant difference ($P<0.01$, SED 0.2372) of mean disease scores between the species of fungi. The mean disease score difference was statistically larger between strains than within strains ($P<0.01$). This result demonstrates that any pathogenicity test may vary depending on the strain of any given fungal species inoculated onto each of the hosts.

II. Preliminary pathogenicity screen of *Cylindrocladium scoparium.*

Another separate preliminary pathogenicity screen was also undertaken for *C. scoparium.* Sixteen isolates obtained from white clover (13 isolates), perennial ryegrass (2 isolates) and subterranean clover (1 isolate), were each grown on PCA at 20°C for 5 days to measure colony growth rates. Each isolate was then inoculated onto one seedling of white clover and perennial ryegrass, which had been germinated axenically on WA.

All seedlings of white clover and ryegrass were killed five days after inoculation by all 16 isolates of *C. scoparium* indicating all strains were similarly pathogenic to these hosts. A single strain (C2R4.2P15S15), isolated from white clover in dairy pasture, was selected for use in the remaining tests as it had the fastest colony growth rate on PCA.

APPENDIX 8: Data sheet used to map and count roots observed using a minirhizotron borescope.

root turn-over rate

RG 082	Tube: C4 Species: clover Rep: 2	Green	YELLOW	RED
Date: 17/7		2	1	3
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
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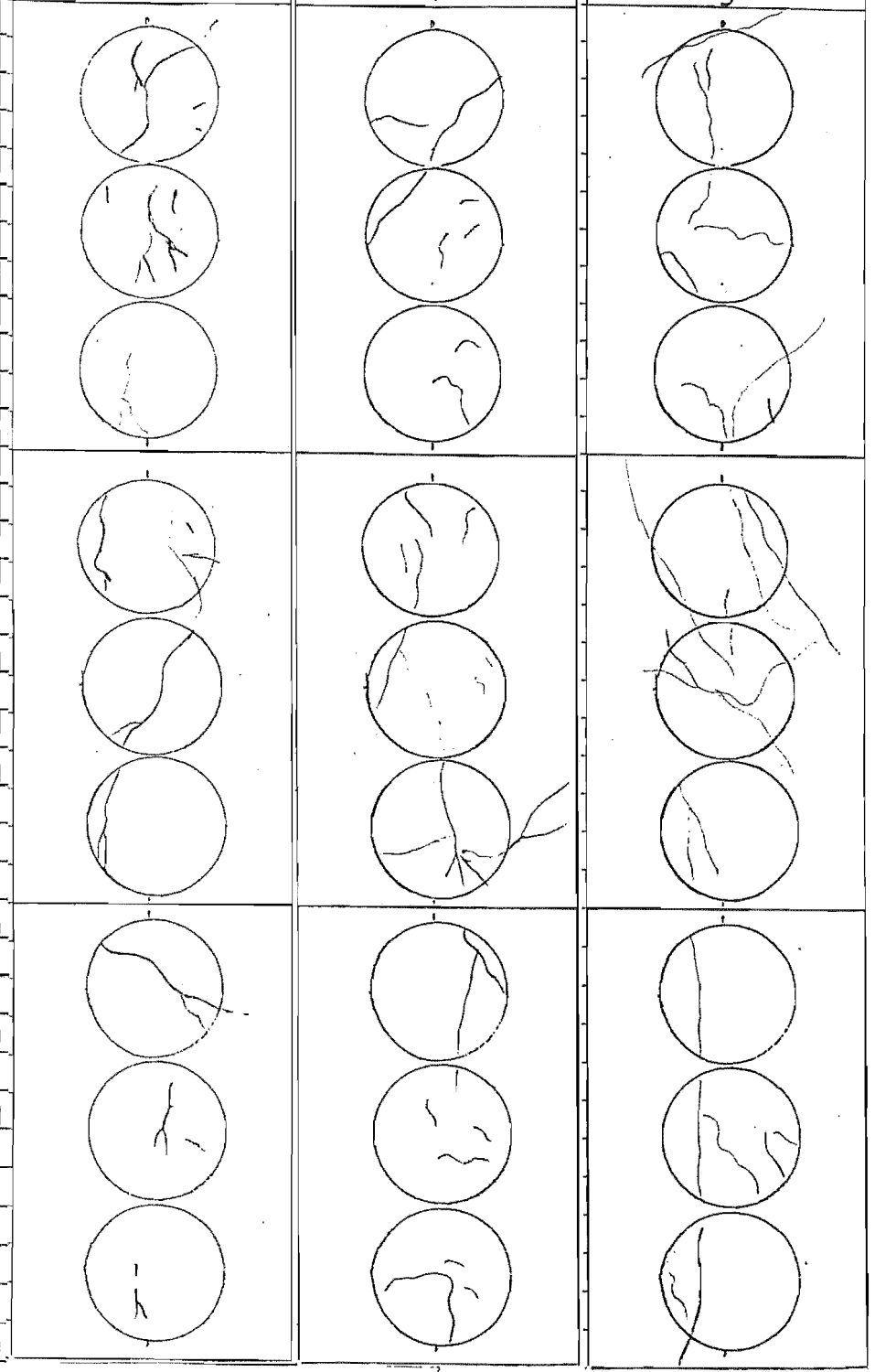
36				



APPENDIX 9: Publications arising from this work

Waipara N. W., di Menna M. E., Cole A. L. J. and Skipp R. A. (1996a) Potential pathogenicity of pasture plant root-colonising fungi to seedlings of legumes and grasses. *Proceedings of the 49th New Zealand Plant Protection Conference.* 49: 212-215.

Waipara N. W., di Menna M. E., A.L.J. C. and Skipp R. A. (1996b) Soil moisture effects on root rot of white clover caused by *Codinaea fertilis. Proceedings of 49th New Zealand Plant Protection Conference.* 49: 216-219.

Waipara N. W., di Menna M. E., Cole A. L. J. and Skipp R. A. (1996c) Pathogenicity of *Cylindrocladium scoparium* to pasture clover and grass species. *Australasian Plant Pathology* 25: 205-211.

Waipara N. W., di Menna M. E., Cole A. L. J. and Skipp R. A. (1996d) Characterisation of *Thozetella tocklaiensis* isolated from the roots of three grass species in Waikato pastures, New Zealand. *New Zealand Journal of Botany* 34: 517-522.

Poster:

August 1994 Australasian Plant Pathology Society Conference, Lincoln, New Zealand.

Pathogenicity of root colonising *Fusarium* spp. against various pasture species.